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# QUARTERLY JOURNAL

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# MICROSCOPICAL SCIENCE.

EDITED BY

SIR RAY LANKESTER, K.C.B., M.A., D.Sc., LL.D., F.R.S.,

HONORARY FELLOW OF EXETER COLLEGE AND HONORARY STUDENT OF CHRIST CHURCH, OXFORD;  
MEMBER OF THE INSTITUT DE FRANCE (ASSOCIÉ ÉTRANGER DE L'ACADEMIE DES SCIENCES);  
CORRESPONDENT OF THE IMPERIAL ACADEMY OF SCIENCES OF ST. PETERSBURG, AND OF THE  
ACADEMY OF SCIENCES OF PHILADELPHIA, AND OF THE ROYAL ACADEMY OF SCIENCES  
OF TURIN; FOREIGN MEMBER OF THE ROYAL SOCIETY OF SCIENCES OF  
GÖTTINGEN, AND OF THE ROYAL BOHEMIAN SOCIETY OF SCIENCES, AND  
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FOREIGN ASSOCIATE OF THE NATIONAL ACADEMY OF SCIENCES, U.S., AND MEMBER OF THE  
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HONORARY FELLOW OF THE ROYAL SOCIETY OF EDINBURGH;  
LATE DIRECTOR OF THE NATURAL HISTORY DEPARTMENTS OF THE BRITISH MUSEUM; LATE PRESIDENT OF THE  
BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE; LATE PHILIPPIAN PROFESSOR OF  
PHYSIOLOGY IN THE ROYAL INSTITUTION OF GREAT BRITAIN.  
LATE LINACRE PROFESSOR OF COMPARATIVE ANATOMY AND FELLOW OF MERTON COLLEGE, OXFORD;  
EMERITUS PROFESSOR OF ZOOLOGY AND COMPARATIVE ANATOMY IN UNIVERSITY COLLEGE, UNIVERSITY OF LONDON.

WITH THE CO-OPERATION OF

ADAM SEDGWICK, M.A., F.R.S.,

FELLOW OF TRINITY COLLEGE, CAMBRIDGE, AND PROFESSOR OF ZOOLOGY IN THE IMPERIAL COLLEGE OF  
SCIENCE AND TECHNOLOGY, LONDON;

SYDNEY J. HICKSON, M.A., F.R.S.,

BYER PROFESSOR OF ZOOLOGY IN THE UNIVERSITY OF MANCHESTER;

E. A. MINCHIN, M.A., F.R.S.,

PROFESSOR OF PROTOZOOLOGY IN THE UNIVERSITY OF LONDON;

AND

GILBERT C. BOURNE, M.A., D.Sc., F.R.S.,

LINACRE PROFESSOR OF COMPARATIVE ANATOMY, AND FELLOW OF MERTON COLLEGE, OXFORD.

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## Part I.

By

**George E. Nicholls, B.Sc., F.L.S.,**

Professor of Biology at the Agra College, University of Allahabad.

(From the Zoological Department, King's College, University of London.)

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With Plates 1 to 5 and 8 Text-figures.

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## I. PREFACE.

IN October, 1907, when working in the Zoological Laboratory of the Imperial College of Science and Technology, I undertook, upon the suggestion of Professor Dendy, an investigation into the structure known as Reissner's fibre. Since January, 1908, the work has been carried on in the Zoological Department at King's College (University of London).

At that time the several papers which had been published some years earlier by Porter E. Sargent, in which he had announced his "optic reflex theory," had attracted considerable attention, and his theory had been accepted by the authors of several text-books, notwithstanding that his results had not received adequate confirmation. In view of the very remarkable character of his observations and his deductions therefrom, confirmation appeared desirable, and accordingly I set out to examine the fibre in a number of vertebrates. From the very outset, however, I found that in several important, and, indeed, fundamental particulars, my observations differed markedly from those recorded by Sargent. I have accordingly refrained from publishing any account of my work until, from an examination of a very large

number of individuals of different species, I should be able to place on a secure foundation results which are so greatly at variance with the "optic reflex theory" of Sargent.

As the result of this investigation, extending now over a period of nearly five years, I am convinced that both in its origin in the brain, and in its ending in the sinus terminalis, the condition of Reissner's fibre is altogether different from the account given of it by Sargent, and I shall hope to succeed in demonstrating that this fibre is not a nerve-tract at all, and that consequently it cannot possibly have the function assigned to it by that author.

The investigation has necessitated the preparation and examination of series of sections of the whole or parts of the central nervous system of between three and four hundred individuals, which have been selected from nearly seventy species. I have also endeavoured to ascertain the function of Reissner's fibre by means of experiments upon living fishes performed at the Laboratory of the Marine Biological Association at Plymouth. The results of these experiments, a short account of which has recently been published ('12), are quite in accord with the suggestion made by Dendy ('09) that the fibre and associated sub-commissural organ form part of an apparatus for automatically regulating the flexure of the body.

The altogether unexpected proportions to which the work has attained have rendered it advisable to publish it in several parts. This, the first part, will be confined mainly to an account of the conditions observed in the Cyclostomes.

The literature list appended does not profess to be complete. It includes only the works referred to in this present part, and the reader is referred to the work of Sargent ('04) for a more complete bibliography of earlier writings which have a bearing, more or less direct, upon this subject.

It is with pleasure that I acknowledge my indebtedness to Professor Dendy, to whom the inception of the work was due, not only for his invaluable advice and criticism during the progress of the work and in the revision of the manuscript,

but also for most kindly undertaking the reading of the proofs. Further, he has placed at my disposal some valuable material, and allowed me the use of his large collection of slides. I wish also to express my thanks to Dr. Allen, Director of the Marine Biological Laboratory at Plymouth, and to his staff, for the facilities afforded me for the collection of material and for the carrying out of experimental work while occupying the British Association's table there.

For assistance in procuring other of the material employed in this research I desire to express my obligations to Professor Meek, Dr. Woodland, and Mr. W. F. Allen, while to Mr. R. W. H. Row my thanks are due for the greater number of the photomicrographs used in illustration, and to Mr. Charles Biddolph for assistance in the preparation of many of the series of sections.

## II. INTRODUCTION.

### (a) Methods and Material.

In the brain and spinal cord of almost every specimen examined Reissner's fibre could be recognised without difficulty. In a few instances only was it apparently absent, and this, practically in every case, in animals which had been preserved entire in fixing fluids of low penetrative power, or where, if the brain-case had been opened, as in some of the larger specimens, there had been insufficient exposure of the brain and spinal cord to the action of the fixing reagents.

In the preservation of such of the material as I was able to obtain in the living condition a number of the commonly employed fixing reagents were tried. Of them all, the aceto-bichromate mixture (Bolles Lee, pp. 49-50), with which I had previously obtained satisfactory results on particularly refractory Teleost material, proved the most generally useful, and was latterly made use of almost exclusively, being found to combine fairly rapid penetrative powers with a good hardening action. With this fluid, too,

there is no risk of overhardening, and the material may be left in it safely for several days, or even weeks, and, if thoroughly washed, is always found to be in good condition for staining. It has, too, the further advantage that small entire specimens left in it for several days usually do not require subsequent decalcification.

Specimens of small size (small ammocœtes, embryo dog-fish, Teleosts up to 30 mm., or even 40 mm. in length, amphibian larvæ, etc.) were, as a rule, plunged whole into the fixing fluid; in the case of larger specimens some dissection was necessary.

Wherever practicable freshly killed material was taken, and the brain partially exposed as rapidly as possible, the entire animal then being plunged into a large quantity of the reagent. Further dissection to complete the exposure of the brain was usually carried on while the specimen was immersed in the fixing fluid.

Such specimens as were sufficiently small were sectioned entire, all risk of damage to the central nervous system being thus avoided. In most cases, however, the length of the central nervous system was too great to admit of the cutting of longitudinal sections through its entirety. In these, the brain (or head) with a considerable portion of the spinal cord behind it, and also the terminal piece of the spinal cord, were removed, but, before the spinal cord was severed, the exposed portions of the central nervous system were allowed to become thoroughly penetrated and fixed. The severance of the spinal cord might generally be safely performed, where aceto-bichromate was the reagent employed, after the lapse of from half an hour to one hour, according to the size of the specimen.

By the adoption of this precaution the characteristic recoil of the fibre, with the resulting tangle of its free ends, which has been remarked by several observers (Sargent, '04, Nicholls, '12), was generally prevented, and the fibre was preserved in its natural relations. In one or two instances, however, it was found that Reissner's fibre had, notwith-



standing, suffered accidental breakage prior to, or during, fixation.

In the case of some of the larger animals, in order to ensure thorough penetration it was found necessary to remove part of the brain on one side. This was cut away with a razor, but only after partial hardening, in order to lessen the risk of crushing together the parts of the brain and the consequent disturbance or displacement of the fibre. Where practicable, however, the brain was preserved entire, the removal of part being found to be necessary principally in the case of mammalian brains.

Many different stains were employed, Reissner's fibre being stained strongly by several of these. It is brought out especially well after fixation in aceto-bichromate by heavily staining in bulk in Grenacher's borax-carmin, followed, upon the slide, by picro-indigo-carmin. This latter stain was prepared by mixing one part of a saturated solution of picric acid in 70 per cent. alcohol with two parts of a saturated solution of indigo-carmin in 70 per cent. alcohol. Sections immersed for about five minutes in this solution become considerably over-stained. This excess of stain is got rid of by washing in a number of changes of 70 per cent. alcohol till all trace of free picric acid is removed. (If the washing be not thorough the residual picric acid begins to crystallise out in a few days.) Sections treated in this way usually show axis-cylinders stained red, with medullary sheaths green, while Reissner's fibre appears of a dull purple tint. Nerve-cells stain variously, the nuclei of all being particularly well brought out, while the cytoplasm, in most cases, is only lightly stained; in the "Dachkern" cells, however, the cytoplasm becomes well stained, and takes on a reddish or purplish tint. Blood-corpuscles are stained green.

If the washing in 70 per cent. alcohol has been prolonged, Reissner's fibre fades to a pale blue or blue-green colour, and connective tissues appear bright blue.

Another stain which has given me good results is iron-brazilin used as described by Hickson ('01). Some especially

satisfactory results have been obtained by the use of this stain with brains of *Petromyzon* which had been fixed in aceto-bichromate, the nerve-fibres being wonderfully defined. It is, indeed, particularly useful for material where the fibres are non-medullated.

Various hæmatoxylin stains were also tried, and generally served to bring out Reissner's fibre well. Ehrlich's acid-hæmatoxylin stains the fibre strongly, and Heidenhain's iron-hæmatoxylin upon aceto-bichromate material in particular yields very satisfactory results. A modification of Weigert's method, suggested by C. J. Herrick, was tried upon material fixed in aceto-bichromate as well as upon material fixed in Flemming's stronger fluid and in Zenker's fluid; in every case Reissner's fibre was found to take the stain strongly, but was readily decolorised by any of the several decolorising fluids commonly employed.

As a test for elastin, both Unna's orcein stain and Weigert's elastin stain, which were brought to my notice by Professor Dixon, were tried upon the fibre in the spinal cord of the frog, and with both reagents the fibre became stained, but lightly.

The methylene-blue stain recommended by Ramsey ('01) was employed upon both Teleost and Elasmobranch brains. It was observed that Reissner's fibre took up the stain quite well, but unfortunately the stain appears to be transient.

The rapid method of Golgi was tried upon Elasmobranch material (*Raia*, *Scyllium*), and although quite satisfactory general impregnations were obtained, I found, as apparently all previous observers have done, that Reissner's fibre invariably fails to become impregnated. This fact has been explained by Sargent as probably due to the presence of much non-dialisable colloid material in the cerebro-spinal fluid which surrounds the fibre, which was supposed to interfere with the action of the fixing fluid. Accordingly, in preparing some Selachian material for this method, I removed about one third of the brain by a longitudinal vertical cut, thus partly exposing the ventricles and allowing the cerebro-spinal fluid to drain away, but Reissner's fibre did not even

then become impregnated. In *Raia*, in particular, it was noted that the cells of the "Dachkern" and of the sub-commissural organ were also unimpregnated, and, for that reason, were conspicuous as yellowish patches upon the almost black background.

A modification of the method of Bielschowski, suggested by Stewart Paton ('07), was tried upon embryo *Scyllium canicula*, and upon a small teleost (*Esox*) brain. In the case of both, successful impregnations were obtained, nerve-fibres being picked out in a deep blue-black, while unimpregnated tissue takes on a soft grey tint. Reissner's fibre appeared homogeneous, and showed no indication of the coloration to be observed in neuro-fibrillæ, but took on the pale grey colour. The cells of the "Dachkern" and the giant cells of the cord became heavily impregnated, the network of neuro-fibrillæ within some of these latter (in embryo *Scyllium*) being wonderfully defined.

The material examined includes:

CYCLOSTOMI.—*Petromyzon fluviatilis*, *Ichthyomyzon* (*Entosphenus*) *tridentatus* (ammocetes only), *Geotria australis*, *Myxine glutinosa*, *Bdellostomacirrhatum*, *B. (Polistotrema) stouti*.

ELASMOBRANCHII.—*Chimæra* (*Hydrolagus*) *eolliei*; *Scyllium canicula*, *Raia* (three species), *Rhina squatinata*.

GANOIDEI.—*Lepidostens osseus* (larvæ).

TELEOSTEI.—Some twenty or more species of the genera *Esox*, *Anguilla*, *Phoxinus*, *Umbra*, *Gobius*, *Blenius*, *Gasterosteus*, *Syngnathus*, *Agonns*, *Cottus*, *Trigla*, *Mugil*, *Rhombus*, *Gadus*, *Labrus*, *Lophius*, *Box*, etc.

AMPHIBIA.—Both adults and larvæ of *Amblystoma* sp., *Salamandra* (two species), *Molge* (two species), *Rana* (two species), and adults only of *Hyla* sp., *Bufo vulgaris*, and *Bombinator igneus*.

REPTILIA.—Two different species of *Gecko*, *Lacerta*

(three species), *Anguis fragilis*, *Pygopus* sp., *Hinulia* sp., *Tropidonotus natrix*, *Emys* sp., *Testudo stellata*, *Sphenodon punctatus* (adult and embryonic).

AVES.—*Gallus domesticus* and *Columba livia*.

MAMMALIA.—*Talpa europæa*, *Erinaceus europæus*, *Mus musculus*, *Microtus arvensis*, *Lepus cuniculus*, *Cavia cobaya*, *Felis domestica* (?), *Anthropopithecus*, and *Homo*.

### (b) Historical Review.

So very complete a survey of the work of the earlier investigators who have noticed Reissner's fibre and related structures has been given by Sargent ('04) that it will be necessary for me here to do little more than consider the more recent papers which refer to this subject, most of which have appeared since Sargent's preliminary paper ('00).

It is now a little more than fifty years since Reissner ('60) announced his discovery of the fibre which bears his name. He described it as a fine cylindrical rod lying freely in the *canalis centralis* of the spinal cord in *Petromyzon*, but he did not ascertain either its origin or ending. He believed it to be a pre-formed structure of a nervous nature.

For nearly forty years after its discovery the fibre remained strangely neglected, and of the comparatively few observers who mention its occurrence the greater number saw it only in the *canalis centralis* of the spinal cord, and appear to have agreed with Stieda ('68, '73) that the structure was an artifact resulting from the coagulation of the cerebro-spinal fluid under the action of certain fixing reagents. Sanders ('94) deserves notice as being the first observer to trace the fibre forwards (in *Myxine*) into the cavity of the mid-brain, and backwards into the *sinus terminalis*. Mayser ('82), with whose work Sanders appears to have been unacquainted, had previously found the fibre in the fourth ventricle of Teleosts.

A new era in the study of Reissner's fibre may be said to

have commenced with the work of Studnička, who, in 1899, announced that he had observed the fibre in a large number of forms. His description, however, relates principally to *Petromyzon*. He discovered the posterior end of the fibre in a tangled condition somewhat similar to that described by Sanders in *Myxine*. He expressed the opinion that it was a pre-formed structure, and stated that he found it to be homogeneous, affording no evidence of internal structure. He concluded that it was non-nervous, and probably produced as a secretion of the ependymal epithelium of the *canalis centralis*. He failed, however, to observe its connection anteriorly with the sub-commissural organ (although, in *Petromyzon*, he succeeded in following it forward to a point immediately beneath the posterior commissure), and he supposed, erroneously, that the anterior end of the fibre was free and possessed of the power of growing forward.

Six months later there appeared the first of Sargent's preliminary papers. In this paper ('00) he states (p. 41) that "the course of Reissner's fibre through the ventricles to its termination anteriorly has been most thoroughly studied in Teleosts, where it has been followed continuously in *Cynoscion*, *Pomatomus*, *Morone*, *Amia* and *Salvelinus*. The fibre has been followed to its termination in the torus also in *Raja*, *Lepidostens*, *Necturus*, *Alligator*, *Scelopoteris*, garter-snake, and less completely in many other species including the mouse and pigeon." (The spaced type is mine.)

It will be sufficient comment upon this statement to recall the fact that the torus longitudinalis is a structure peculiar to the brain of bony fishes, as was shown by Rabl-Rueckhard long since ('84). What Sargent supposed to be the torus longitudinalis, and labels as such in his text-fig. 1, is evidently the posterior commissure.

Describing the course of Reissner's fibre he says (op. cit., p. 42): "Passing along the median fissure of the torus for one half to two-thirds its length and close to its surface (figs. 8, 9), the fibre passes beneath the membrane which



covers the torus and enters the brain substance (fig. 10). In *Cynoscion* and *Salvelinus* the fibre, after passing beneath the membrane, may be followed for  $100\mu$  or more before it breaks up." The "membrane" here referred to, in the light of his text-fig. 1, is indubitably that well-defined tract of highly modified ependymal epithelium beneath the posterior commissure for which the name "sub-commissural organ" has been recently suggested (Dendy and Nicholls, '10).

Sargent further seems to have confused the cavity of the mid-brain with the third ventricle, for he says (p. 39): "As already stated, Reissner's fibre extends through the whole length of the *canalis centralis* of the cord and continues cephalad through the fourth and third ventricles to the anterior end of the optic lobes."

In an appendix to this paper he controverts Studnička's ('99) statements (published six months earlier), and reaffirms his own opinion that Reissner's fibre is a pre-formed structure of a nervous nature.

His conclusions were criticised by Kalberlah ('00) in the same year. This author figures Reissner's fibre in a transverse section of the spinal cord of an embryo of *Acanthias*, and from a study of the condition of the fibre in that embryo he comes to the conclusion that it is an artifact. It is quite possible that what he describes as "*eine ganze Kollektion solcher Fadenquerschnitte*" was simply a tangled mass of fibre, which might easily present such an appearance in transverse section.<sup>1</sup>

Although he actually quotes the sentence from Sargent's paper in which that author refers to the posterior commissure under the name "torus," Kalberlah appears not to have noticed either this or other errors in Sargent's work, to which I have here called attention. Sargent subsequently ('04, p. 135) dismissed Kalberlah's criticism as valueless, remarking that that author had probably himself not seen

<sup>1</sup> I have seen appearances precisely similar to that figured by Kalberlah in sections of the spinal cord of the mouse in which a tangled heap of Reissner's fibre was cut through.

Reissner's fibre because in embryos of *Acanthias* at that age the fibre is not developed.<sup>1</sup>

In this year another paper containing a reference to Reissner's fibre was published by Studnička ('00). Replying to Sargent's criticism of his former work, he remarks that that author's statements require confirmation, and further, that he himself found it very difficult to reconcile the idea of the fibre being a nervous structure with his own observations upon its relations to the *Ventriculus terminalis*.

This paper is also noteworthy in that it contains the earliest careful figures of the modified ependymal epithelium of the sub-commissural organ (in the lamprey and dogfish). In his text the author states that he was unable to determine the function of this epithelium, and he does not appear in the least to have realised its connection with Reissner's fibre.

In the following April Sargent ('01) published a second preliminary paper, dealing this time with the development of the fibre, which, he said (*op. cit.*, p. 445), "has been studied in about twenty different species, and has been more or less completely worked out in representatives of all the chief groups of vertebrates." His descriptions, however, relate only to Cyclostomes, Ganoids and Selachians, in which he claimed to have discovered "axons" which, growing out from numerous conspicuous nerve-cells (the "Dachkern" of Rohon) in the tectum mesencephali, emerged into the aqueductus sylvii either immediately (*Amia* and *Petromyzon marinus* [?]), or after passing forward in the brain-tissue to the anterior end of the tectum (Selachians), these axons uniting in both cases in the aqueductus Sylvii to form Reissner's fibre. Referring to the condition in Selachians he says ('01, p. 448): "Where Reissner's fibre leaves the brain tissue the membrane covering of the roof of the ventricle is continued in a cone-like projection surrounding the fibre

<sup>1</sup> Kalberlah, so far as I can find, nowhere states either the age or the size of the embryo in question. I, myself, find the fibre developed in the closely related *Scyllium canicula* at a very early stage, and Sargent also records its existence early in development in *Mustelus*.

(fig. 9).” This “cone-like projection” is obviously the posterior end of the sub-commissural organ (cf. text-fig. 2, A, B), and this constitutes his only reference in this paper to this organ.

Sargent apparently still continues in this paper to confuse the aqueductus Sylvii with the third ventricle, for he says (p. 448): “In the adult *Petromyzon* Reissner’s fibre passes through the canalis centralis and the fourth ventricle, from which it enters the brain-tissue of the basal portion of the cerebrum, and passing through this emerges into the third ventricle. Here it breaks up into several trunks and continues forward to the anterior portion of the ventricle, where, after further division, it enters the tectum.” The “basal portion of the cerebrum,” too, is somewhat vague, but Sargent means apparently the postero-ventral part of the optic lobes, and perhaps also of the cerebellum.

Sargent further describes (’01) in the sinus terminalis of *Amia*, *Raja erinacea* and *Petromyzon marinus*, free “posterior canal cells,” which, he states, send forward “axons” to meet the backwardly growing Reissner’s fibre, and says (p. 447): “The fibre, then, is a nerve-tract composed of axons running in opposite directions, both cephalad and caudad. The development of this apparatus as outlined for *Amia* is typical for all vertebrates” (my spaced type).

It is in this paper that Sargent, upon what appears to me to be altogether unsatisfactory and insufficient evidence, first puts forward the optic reflex theory, which he formulates as follows (p. 450): “The apparatus which is the subject of this paper forms, I believe, a short circuit between the visual organs and the musculature, and has for its function the transmission of motor reflexes arising from optical stimuli.” His text-fig. A (p. 449) offers a diagrammatic representation of the structure of this “optic reflex apparatus,” but he wholly omits, so far as I can find, to make clear in what way he supposes the alleged axons of the “posterior canal-cells” to be related to the cells of the “Dachkern” or other brain centre.

Sargent states also ('01, p. 451) that "in those species which are totally blind no trace of this apparatus is to be found"—an erroneous statement that has since found its way into several text-books, notwithstanding that, so long ago as 1894, Sanders had mentioned the existence of a particularly well-developed Reissner's fibre in *Myxine*, his discovery being confirmed by Studnička in 1899.

In the latter part of the same paper Sargent gives an account of certain experiments performed upon living sharks, the operation consisting in breaking Reissner's fibre in the fourth ventricle. He states that he observed that "those sharks in which the fibre had been broken showed a slowness in response to optical stimuli," indicated by an inability to turn quickly to avoid obstacles interposed suddenly in their paths. This is construed as evidence that the breaking of Reissner's fibre had interrupted the conduction of optical stimuli by the "short-circuit" path constituted by that fibre. I have pointed out elsewhere ('12) that the evidence afforded by these and other experiments is susceptible of another and much simpler explanation in accordance with Dendy's suggestion ('09), to which I shall have to refer shortly.

Later in the same year Houser ('01) published an account of the neurons and the supporting elements of the Selachian brain, his descriptions being based upon the study of adult *Mustelus*. In this memoir he repeatedly states that he found Reissner's fibre arising as the product of the coalescence of paired fibre-tracts, the constituent fibres of which were the axons of the cells of the "Dachkern," or roof nucleus.

This account of the fibre in *Mustelus* agrees closely with the condition in Selachians as at that time described by Sargent from a study of the embryo of *Raja*, but differs in an all-important particular from the account of the fibre in *Mustelus* and Selachians generally as given subsequently by Sargent (1904), when the fibre was (correctly) described as continuing forwards beneath the posterior commissure.

Houser apparently did not notice (as, indeed, extraordinary as it may appear, no subsequent observer seems to have done)

the startling mistakes in the earlier of the two papers by Sargent, with whose work he appears so entirely in agreement.

In 1901, Cole and Johnstone ('01) drew attention to the occurrence of Reissner's fibre in the *canalis centralis* of *Pleuronectes*, and appear to have accepted Sargent's theory without question.

Dendy, early in 1902, unaware that it had been previously described or figured, directed attention to the sub-commissural organ, which he spoke of as a "pair of ciliated grooves." He noted the occurrence, in the ammocœtes of *Petromyzon* and *Geotria*, of a pair of conspicuous grooves lined by long columnar epithelium, the free border of which was beset with short cilia. These grooves were described as beginning at the posterior limit of the habenular ganglia, and extending backwards beneath the posterior commissure to the hinder end of that structure. He suggested that these grooves were concerned in promoting the circulation of the cerebro-spinal fluid.

Kölliker ('02) announced that he had observed Reissner's fibre in members of several different classes of vertebrates, and admitted that it must be accepted as a pre-formed structure to be found in all classes of vertebrates from the birds downwards. He figured the fibre in transverse section in the spinal cord of *Protos* and *Siredon*, and called attention to the widely divergent views upon the nature of the fibre which had recently been expressed by Studnička, Sargent and Kalberlah.

In 1903 there appeared a short paper by Sargent ('03) dealing with the endymal groove (sub-commissural organ). He stated that this serves merely as an attachment plate or anchorage for Reissner's fibre, and omitted all reference to Dendy's paper ('02). He remarked that this structure, while present in all vertebrates, is conspicuous in Cyclostomes, Selachians and reptiles, less prominent in Teleosts, birds and amphibia, and inconspicuous in mammals. As has since been shown (Dendy and Nicholls, '10), the last part of this statement (if we except the Primates) is entirely erroneous.

In the same year still another work by Sargent appeared ('03A), dealing primarily with the torus longitudinalis, which he this time correctly identified. In this he gives a full historical survey and a description of the condition of this organ in a number of Teleosts. He points out the several errors into which different observers have fallen in attempting to homologise this structure, peculiar to Teleosts, with various parts in the brains of other vertebrates, but appears completely to overlook the fact that he himself had confused the torus longitudinalis with the posterior commissure! He accepts Rabi-Ruckhard's ('84) disproof of the earlier theory that the torus is the homologue of the fornix of higher vertebrates, but denies, rightly enough, that the latter author is correct in supposing that it is homologous with the sub-commissural organ ("ependymal thickening"), pointing out that in Teleosts both of these structures co-exist.

Sargent himself, however, falls into fresh error, as I believe, in stating that the torus is the homologue of the "Dachkern" or roof-nucleus of other vertebrate classes. He claims that he has succeeded in tracing from the cells of the torus three separate fibre-tracts strictly homologous with the three fibre-tracts which he had already traced from the "Dachkern" in Selachians.

I cannot believe that this suggested homology can be correct and, on the contrary, I shall bring forward evidence later in this paper to show that the "Dachkern" nucleus is also present in some Teleosts, and is perfectly distinct from the torus.

In 1904 Sargent published what must be regarded as his principal paper ('04) upon Reissner's fibre, in which he collected and amplified the results announced in his earlier works.

In a footnote (p. 153) he refers to Dendy's ('02) description of the "ciliated grooves" in the brain of the ammocœtes of *Petromyzon* and *Geotria*. He represents that author as stating that the cilia of the grooves are longer than those



occurring on the general ventricular (ependymal) epithelium, whereas what Dendy said was really precisely the opposite. He then goes on to say—"I believe that there are no cilia in the grooves." I shall show later that the grooves are abundantly ciliated.

He, again and more emphatically, on the strength apparently of an examination of a single series of sections through the tail of *Petromyzon marinus*, controverts ('04, p. 160) the account given by Studnička ('99) of the ending of the fibre posteriorly in *Petromyzon* and *Myxine*, and appears to be wholly unaware of Studnička's reply ('00) to his earlier criticism. From his own account and figure ('04, Pl. I, fig. 8) it is evident that Sargent himself had not seen the actual termination of the fibre in the sinus terminalis of the adult lamprey.

In this paper, also, he institutes a comparison between Reissner's fibre and the large (Dachkern) cells from which he says it arises, and the giant fibres and cells of *Amphioxus* and *Chaetopoda* (p. 158), and concludes—"It is possible . . . that Reissner's fibre and the cells which give rise to it are represented by elements in the invertebrate nervous system."

To avoid repetition it will be desirable to postpone a more detailed criticism of Sargent's general results, and also of those of Houser, until after I have given a general account of what I believe to be the true relationships of the parts concerned. Certain other more special criticisms will follow the description of each class.

At the end of his memoir Sargent announced that a second part, dealing with the higher vertebrates, was already well advanced (June, 1904), and would, it was hoped, appear in about a year. This has not yet been published, but in the interval that has since elapsed several references to this subject have appeared in the works of other authors.

Kolmer ('05), in an elaborate paper on the spinal cord of *Ammonoetes*, published in the following year, records the finding of Reissner's fibre in the *canalis centralis* of that

animal, but states that, although he was able to follow it forwards into the cavity of the mid-brain, he could determine neither its origin nor its ending. He disagrees with Sargent as to its nervous nature, and inclines rather towards Studnička's view of its origin and character. In his summary he states (p. 209): "Der Centralkanal enthält konstant den Achsenfaden (Reissner'sche Faden). Es ist sicher kein nervösen Gebilde, wahrscheinlich ein Sekretionsprodukt der Ependymzellen."

In the same year Sargent's theory was accorded a place in vol. ii of Sedgwick's 'Text-Book of Zoology' ('05), where it is stated (p. 195) that Reissner's fibre "consists of a bundle of nerve-fibres and communicates with the tissue of the spinal cord throughout its length. It appears to be absent in blind fishes."

Dendy, in 1907, described and figured the fibre in *Geotria australis*, calling attention to its relation to the sub-commissural organ (ependymal groove). He suggested a possible connection with the pineal eye, a view which he has since abandoned, and, while accepting Sargent's theory as to the nature and function of the fibre, said (p. 15): "I find it difficult to believe that such a remarkable and well-developed structure as the ependymal groove should be required solely for the function which Sargent assigns to it."

In the same year (1907) Sargent's work obtained notice in several text-books. Johnston ('07, p. 148) put forward the optic reflex theory with a certain reserve, but, so far as I can find, his work contains no reference to the sub-commissural organ! Sherrington ('07) apparently accepted the optic reflex theory freely, and in the English edition of Weidersheim's text-book ('07) it also finds a place, though only in a footnote.

In 1908 I announced in 'Nature' ('08) the presence of Reissner's fibre in the frog, and called attention to its relation to the sub-commissural organ in that animal.<sup>1</sup>

<sup>1</sup> I have since found that the fibre had previously been seen in the *canalis centralis* of *Rana* by Stieda ('70), although he did not refer to it by name, and considered it to be an artifact.



In March of the same year, Ayers ('08) described the occurrence of "ventricular fibres in Myxinoids." His descriptions, though somewhat vague, appear to relate to Reissner's fibre, and to confirm, to a large extent, the account of that structure as given in 1894 for Myxine by Sanders, of whose work, as, indeed, of the existence of this fibre in other vertebrates, Ayers seems, however, to have been wholly unaware.

Edinger ('08), who some year earlier had given a very diagrammatic figure of the sub-commissural organ of Scyllium, which he considered to be of a secretory nature, is, I believe, the only recent worker to adhere to Stieda's view that Reissner's fibre is to be looked upon merely as an artifact.

In the same year Horsley ('08) recorded the existence of the fibre in Primates, finding it present in two species of *Macacus*. He mentioned certain experiments, performed by himself and Dr. McNalty, and stated that division of Reissner's fibre was not followed by that degeneration of the distal portion which is characteristic of severed nerves, and he expressed the opinion that Sherrington had been premature in accepting Sargent's theory, lacking, as it did, adequate confirmation.

During 1908-1909 Favaro ('08, '09) referred several times to Reissner's fibre. He summarizes Studnička's work and briefly mentions Sargent's "optic reflex theory," concerning which he offers no opinion. He refers also to Ayers' paper, and reproduces a diagram from Kolmer's work in which Reissner's fibre is shown.

In 1909 Dendy ('09) further announced the occurrence of Reissner's fibre in the cat and the tuatara. He altogether repudiated his former acceptance of Sargent's theory, and put forward an entirely new suggestion as to the function of the fibre and the related sub-commissural organ, pointing out that the fibre may quite possibly play merely a mechanical part in automatically regulating flexure of the body, its variations in tension acting as stimuli upon the cells of the sub-commissural organ to which it is attached.

At the same time I myself ('09) published further evidence of the remarkable elasticity of this fibre, its behaviour in the case of specimens of *Bufo* and *Petromyzon* being instanced. I stated definitely that the fibre was not a nerve-tract, and that the suggestion put forward by Dendy as to its function was quite in accordance with the facts so far as they were then known.

In the following year, in a joint paper (Dendy and Nicholls '10), we showed reason for believing that Reissner's fibre, while present almost, if not quite, without exception in the vertebrate series from Cyclostomes to Primates, may prove to be absent in man. We proposed the name "sub-commissural organ" for the "ependymal groove" with which the anterior end of Reissner's fibre is connected, and pointed out that this organ, far from being "inconspicuous in mammals" as Sargent asserted, is in reality a very conspicuous structure in the lower members of this group (mouse, cat). It is, however, less conspicuous in the chimpanzee, and, while well developed in the fœtal human subject, has become reduced to a mere vestige (the "mesocœlic recess") in the adult man.

Still more recently, Dendy (1910), in an account of the pineal organs and adjacent parts of the brain of the tuatara, has given a short account of Reissner's fibre and the sub-commissural organ in that animal.

In the present year ('12) I have given a short description of the condition of Reissner's fibre in the sinus terminalis of several Elasmobranchs, and described certain experimental work carried out by me upon living fish in an attempt to ascertain its function.

(c) General Introductory Account of the Actual Relations of Reissner's fibre and the Sub-commissural Organ.

As is well known, one of the most constant features—I might almost say the most constant feature—in the roof of the brain of all vertebrates is that tract of transversely coursing

nerve-fibres situate at the junction of the fore- and mid-brain. This tract, which is known as the posterior commissure, is by most authors considered as belonging to the mid-brain, of which it is said to mark the anterior boundary. It makes its appearance in development at an extremely early stage, in that downfolding of the roof (the *plica meso-prosencephalica*<sup>1</sup>) which separates the first brain vesicle from the second. Arising at the same time, or perhaps even earlier in development, is another equally constant but little-known structure, the "sub-commissural organ." This is a conspicuous, longitudinal, paired tract of epithelium, produced by a modification of the ependymal epithelium of the brain ventricle on either side of the mid-dorsal line beneath the meso-prosencephalic fold. The ordinary, almost cubical cells of the ependymal epithelium become in this region enormously elongated and fibre-like. Their nuclei mostly pass inward towards the end remote from the brain ventricle, and the whole structure bears a striking resemblance to the epithelium of a sense-organ. From the inner (deeper) end of the cells neuroglial fibres pass, which, collecting into bundles, radiate towards the *membrana limitans externa* on the upper surface of the brain. The ventricular ends of the cells are beset with short cilia.

In the adult, in almost every case, the ependymal epithelium of the sub-commissural organ passes gradually, in the infundibular recess, into the more typical epithelium of the epiphysial stalk.

The paired nature of this sub-commissural organ remains apparent throughout life in certain forms (e.g. *Petromyzonidae*), but in the greater number of cases there is a confluence of the two tracts along their mesial borders, so that the structure takes on the form of a median plate of columnar cells, and under such circumstances may retain traces of its originally paired character only at its anterior and posterior ends. The shape of this plate is variously

<sup>1</sup> *Plica meso-thalamencephalica* would seem to be a better term for this fold.—A.D.

modified in the different classes of vertebrates, being dependent upon the size and relations of the structures of the mid-brain immediately adjacent. Thus, among the forms that I have examined, the sub-commissural organ appears, in transverse section, as a decidedly horse-shoe-shaped structure in Selaehians, reptiles and many mammals (Pl. I, figs. 2, 3, 6, 8, *s.c.o.*), in birds it is strongly arched (Pl. I, fig. 7), and in amphibians (Pl. I, fig. 5) perhaps somewhat less markedly curved, while in many Teleosts it persists merely as a flattened median plate which shows scarcely any trace of its original paired character (Pl. I, fig. 4).

The shape of the sub-commissural organ may be still further modified by transverse folding. In some cases, too, this tract of specialised epithelium extends around the posterior border of the posterior commissure on to the dorsal (posterior) surface of that structure, and may even line a small median anterior extension of the optocœl (the mesocœlic recess) directly above (behind) the posterior commissure (Text-figs. 2, B, and 3, A, *m.r.*).

In man, as was pointed out by Dendy and Nicholls ('10), the mesocœlic recess is the sole vestige of this apparatus. It is connected, in foetal life, with a typical sub-commissural organ, which lies perfectly normally beneath the posterior commissure and bears distinct evidence of its paired origin, but it appears subsequently to lose its connection with the general cavity of the iter, and that part of the sub-commissural organ which lies beneath the posterior commissure altogether disappears.

Between the cilia of the sub-commissural organ are found slender fibrillæ which are distinguishable from the ordinary cilia simply by their greater length. These collect together to form delicate strands which unite to constitute Reissner's fibre. In longitudinal section of the typical brain (compare Text-fig. 5) the fibre is thus to be made out arising well forward from near the anterior border of the sub-commissural organ in the infra-pineal recess. It there lies closely against the surface of the sub-commissural organ near the middle line,

and continues to receive fresh accessions of strands of united fibrillæ along the entire length of the posterior commissure, until it passes caudally from the hinder border of that structure. Thence it stretches as a taut thread lengthwise through the mesocel, usually coming to lie close against the ventral surface of the rhombo-mesencephalic fold. Upon this surface there is almost invariably a median longitudinal groove, which I have termed the "isthmic canal" (Text-fig. 5, *i.c.*). This appears to become deeper with age. It is lined by an ependymal epithelium that differs from the general ventricular investment, the cells being more elongated and staining very strongly. In this groove the fibre lies freely, and, so far as my observations go, never becomes embedded in the brain-tissue (as has been asserted). The isthmic canal is usually deepest at its anterior end, becoming continually more shallow posteriorly till it fades out, and Reissner's fibre, emerging from its hinder end, may be readily traced backwards through the fourth ventricle as a tightly stretched thread which enters the *canalis centralis* of the spinal cord.

The ependymal cells which line the *canalis centralis* are furnished somewhat sparsely with short cilia. Here and there, however, at comparatively short intervals, slightly longer cilia may be made out, which appear to be attached to Reissner's fibre (compare fig. 56). It is, of course, extremely difficult, if not impossible, to determine with absolute certainty that these cilia are not simply lying in contact with the fibre or even glued to it by a coagulum of cerebro-spinal fluid. An examination of a very large number of sections, however, leads me to believe that many, at any rate, of them are indeed actually fused with Reissner's fibre and probably form an integral part of it. Whether, however, they are quite short cilia fused to the fibre only by their tips and serving merely as supports and stays for it, or whether they are of a similar character to the long cilia-like processes of the cells of the sub-commissural organ, I am quite unable to determine, but I suspect that they are of this latter class, and that they

probably take an important part in the growth of the fibre, and may extend backwards in it for an appreciable distance. The fibre extends along the entire length of the *canalis centralis*, maintaining a practically uniform thickness in this part of its course, although, perhaps, diminishing slightly in diameter near its posterior end.

Towards the hinder end of the body the spinal cord undergoes a considerable diminution in size, and tapers off into the delicate *filum terminale*. This becomes a simple epithelial tube, and may, in some fishes, pass beyond the enclosing vertebral canal, to lie unprotected, except for its meninges, just beneath the skin. At its actual extremity the *filum terminale* becomes somewhat dilated, and contains an ovoid enlargement of the *canalis centralis* which is known as the *sinus (ventriculus) terminalis* (fig. 40, *s.t.*). The *sinus terminalis* is, however, only incompletely surrounded by the enlargement of the end of the *filum terminale*, for at about the middle of the sinus the ependymal as well as the nervous elements of the cord disappear, so that the *canalis centralis* actually opens by a wide aperture, the "terminal neural pore," into a lymph-space that is morphologically continuous with the perineural spaces. The posterior wall of the *sinus terminalis* is thus formed only by the fibrous sheath of the spinal cord, which appears to consist of united pia and dura mater. From the *canalis centralis* Reissner's fibre passes through the terminal neural pore, behind which point in many cases, if not in all, it expands into a conical "terminal plug" (fig. 51, *t.p.*), which passes into, and blends with, the connective tissue of the meningeal portion of the wall of the *sinus terminalis*.

The actual fibre is an extremely tenuous thread, whose normal diameter in the adult condition in the greater number of species which I have examined is between one and three micra. In well-hardened material it is almost always distinctly brittle,<sup>1</sup> but in life or in the fresh state in recently

<sup>1</sup> In a single case (*Geotria*) the fibre appears to have retained, to a great extent, its flexibility even in Canada balsam.



killed material it must possess a remarkable degree of elasticity. It would appear to exist in life under considerable tension, and, being subject to continually varying strain with every alteration of the position of the long axis of the body, it must be exceedingly liable to accidental breakage. In such an event, or in the case of artificial section in the fresh condition, the elasticity of the fibre brings about a sharp recoil of the broken ends to form large masses or snarls, as shown, for example, in fig. 19. Even when partially fixed by reagents, however, the resilience of the fibre is such that it will still contract if severed. Under these circumstances the retraction appears to be a comparatively gradual one, and the fibre will then be found twisted into a more or less regular spiral (fig. 16), which is often extraordinarily reminiscent of the retracted stalk of a *Vorticella*. Even where the recoil has been an abrupt one and a large knotted mass has formed, a careful examination practically always discovers the existence of such a spiral winding of the fibre (figs. 15, 19). These knots thus have the character of tangled heaps such as would result from the continued twisting in one direction of one end of a thin elastic thread of which the other end is held fast. In every such case there is a marked decrease in length, and an accompanying and very considerable increase in the diameter of the fibre.

Seen in transverse section, Reissner's fibre seems to show a very thin outer sheath investing an apparently homogeneous central core which possesses a very high refractivity. I believe that this thin dark encircling rim is nothing but an optical effect consequent upon the difference of refractivity of the fibre and that of the surrounding medium. That it is a medullary sheath of myelin, as Sargent declares ('04, p. 145), I can find no reason for believing.

According to that author the central part of the fibre shows, in transverse section, a punctate appearance, which he interprets as the effect of the cut ends of the constituent axis cylinders ('04, p. 146). As stated above, I find no trace of any such structure, and if the fibre is, as I believe, the result

of the coalescence of hypertrophied cilia, the fusion is so intimate that the whole fibre has a homogeneous glassy appearance. In favour, moreover, of such a view of the nature of the fibre is the extraordinary rapidity with which it disintegrates at death. It is practically useless to examine material for Reissner's fibre if that material has been dead for an hour or so before fixation, and further, even in material which has been fixed at death, but which is of sufficient size to prevent speedy penetration, or even in small material where a slowly penetrating fluid has been employed, it is rare to find the fibre preserved. In such cases the general ependymal cilia, too, have almost invariably disappeared.

Apart, then, from the doubtful existence of a delicate investing sheath, I have been unable to demonstrate any internal structure in Reissner's fibre, even with the aid of very high powers of the microscope. It seems certain, nevertheless, in view of the constancy of the mode of recoil which the fibre exhibits, that some very definite internal structure must exist.

An explanation may perhaps lie in the manner of development and growth of the fibre. It may well be that when, in early development, the fibre (arising from fused sub-commissural fibrillæ) grows backwards to enter the *canalis centralis*, it is at once joined by similar fibrillæ or cilia from the ependymal cells, which, overlying the earlier formed structure, continue its backward growth. These, in their turn, would be continually covered by fresh accessions, and the entire fibre would resemble a species of "hay-rope." If the whole of these fibrillæ have a slight spiral growth the manner of recoil of the fibre formed by their union would be easily understandable. Even the retention, to a certain extent, of their individuality by the fibrillæ might in itself be sufficient for the spiral retraction. In all such cases of recoil, however, the fibre has torn itself free from its attachments.

I have already suggested that accidental breakage of Reissner's fibre may occur not infrequently, perhaps, in life, and I have obtained a considerable amount of evidence of



the occurrence of this phenomenon in Cyclostomes and fishes. It will be obvious that the sudden recoil of the broken ends which normally occurs, and which results in the formation of a large coiled knot, must be of use<sup>1</sup> in checking the further retraction of the broken fibre, for this knot blocks the comparatively small lumen of the *canalis centralis*, and, presumably, enables the fibre to establish a temporary hold until regenerative processes shall have restored its connection with the wall of the *sinus terminalis*. That regeneration does occur I have ample evidence to show (cf. Nicholls, '12). The large percentage of cases in which the fibre is found broken and recoiled may be due to the exceptionally strenuous exertions of the animals in their efforts to avoid capture causing the fibre to snap.

#### (d) Critical Discussion of the Views of Sargent and Honser.

Before passing on to a detailed account of Reissner's fibre and the sub-commissural organ in the several vertebrate groups, it will be necessary to discuss certain general statements made by Sargent, some of these being said to be confirmed by Honser.

To the former author, and, though perhaps in a lesser degree, to Studnička, is due the credit of having established the fact of the pre-formed nature of the fibre. Sargent further demonstrated the constancy of its relation to the anterior part of the roof of the mid-brain, but, unfortunately, the mistakes in his earlier papers ('00, '01) (especially those consequent upon his failure, at that time, to recognise the posterior commissure, which, as I have shown above, he supposed to be the *torus longitudinalis*), have coloured all his later work, detracting greatly from its value; for, although subsequently both posterior commissure and *torus longitudinalis* were correctly identified ('03, '04), and the

<sup>1</sup> We have an analogy in the formation of a blood-clot operating to close a severed blood-vessel and so to check further blood-flow.

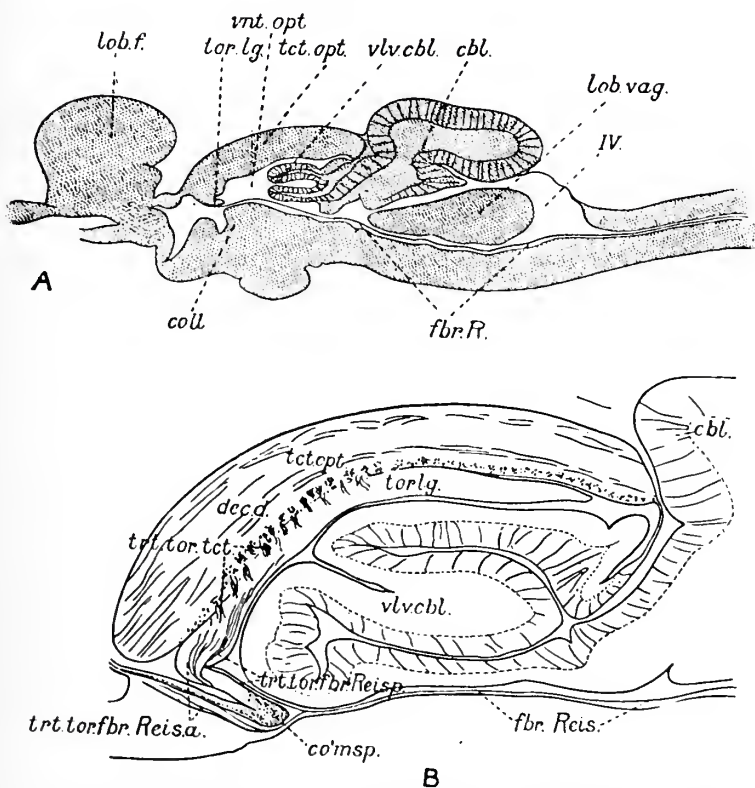
sub-commissural organ (ependymal groove) described in some detail ('03A), Sargent still clung ('04) to the idea that, at any rate in part, Reissner's fibre is traceable directly into the torus, notwithstanding that his original description of such relation of the fibre to the torus was based upon an erroneous identification of this structure. It is clear from his descriptions and also from his text-figure ('00), making allowance for the mistaken identification of the parts concerned, that Sargent actually saw Reissner's fibre lying in its proper position as a single thread below the posterior commissure (termed by him "torus longitudinalis") for one half to two thirds of the length of that structure, and then breaking up to join the sub-commissural organ ("the membrane which covers the torus"). In these earlier descriptions he nowhere suggests a division of the fibre into two main branches.

In his last paper, however, he shows the fibre composed of two main factors—(1) an anterior branch which lies beneath the posterior commissure and which is indisputably the fibre originally described by him as solely constituting Reissner's fibre, and (2) a posterior branch which was not indicated in any way in either of his preliminary papers ('00, '01), but which is now stated to pass above (posterior to) the posterior commissure, and is described as emerging into the mesocoel directly from the torus longitudinalis. This posterior branch is said to be composed of numerous axons, derived, in Teleosts, from the cells of the torus, which he therefore homologises with the "Daehkern," from the cells of which he derives the constituents of the posterior branch in other types.

Sargent does not, however, call attention to this marked discrepancy between his later account and that previously given, and, in the absence of any definite statement to the contrary, the reader is naturally led to suppose that the posterior branch described in 1904 as directly related to the torus is identical with the entire fibre erroneously described in the preliminary paper ('00) as having the same

relations. To render intelligible this somewhat involved matter, I have reproduced in Text-fig. 1 two of Sargent's figures, one (A) being taken from his preliminary paper,

TEXT-FIG. 1.



An exact reproduction of two of Sargent's figures to show the difference between his earlier ('00) and later ('04) accounts of Reissner's fibre and its relations to structures in the mid-brain. The first (A) is taken from his earlier paper ('00) and shows the course of Reissner's fibre correctly, but the posterior commissure is identified (erroneously) as the torus longitudinalis (*tor. lg.*). In B, taken from his latest work ('04), the posterior commissure (*co'msp.*) and torus longitudinalis (*tor. lg.*) are correctly identified, but a second branch of Reissner's fibre has been added, to which the description originally given of the piece ventral to the posterior commissure is now made to apply.

and representing a sagittal section through the brain of a teleost, *Cynoscion regale* ('00, p. 39, fig. 1), the other (E) from his latest paper, representing a similar section through the brain of another teleost, *Pomatomus saltatrix* ('04, p. 209, fig. 1). The brains of both of these fishes had been studied by him when the preliminary paper was written, where it was said of Reissner's fibre ('00, p. 41) that—"its course is the same in all except in so far as it is dependent on the size and relations of the ventricles and other parts of the brain, which differ in different species."

According to my own observations there never is a posterior branch connected with either the torus or the "Dachkern" such as Sargent describes, and one is forced to the conclusion that the introduction of this branch in the later paper (where its course and relations are described in terms practically the same as those applied erroneously to the entire fibre in the first instance) has been due to his neglect to put right the original mistake in identifying the posterior commissure as the torus.

This impression may be a mistaken one, but it is of the utmost importance to emphasise the non-existence of the posterior branch, as it is upon its alleged presence that Sargent's statements as to the connection of Reissner's fibre with the cells of the torus and the "Dachkern" and the consequent homologising of these two structures principally depend.

The anterior branch, which, so far as my own observations go, alone exists, and which, as explained above, was alone figured and described by Sargent in his preliminary paper, is, therefore, in his later work, necessarily described as having (in forms other than Teleosts) quite different relations.

In Teleosts it is traced into the torus longitudinalis by a somewhat devious path through the "Schaltstück" (pars intercalatus) and the posterior commissure, the adoption of such a route being explained by Sargent as due to the early development of the cells of the optic reflex apparatus, which "send their axons into the ventricle from the median plane of the roof (Pl. 7, fig. 47; Pl. 8, figs. 55, 56, 58, *fas.*

*Reis.*) even before the posterior commissure has developed. In the later development the fibres of the posterior commissure, in making their way across from the opposite sides, pass posterior to these axons. Such a pre-posterior commissural tract—the tractus toro-fibræ Reissneris anterior—is found only in Teleosts" ('04, pp. 198-199).

Almost all his figures ('04), however, not only for Teleosts but also for Cyclostomes and Elasmobranchs, show Reissner's fibre arising, either principally or entirely, from a point far forward beneath the posterior commissure. In explanation of this condition Sargent suggests that in these forms the anterior branch is probably derived from fibres which issue from cells in the habenular ganglia, and he attempts to explain, too, in this way, the occurrence of the fibre in blind animals (e.g. *Myxine*, *Amblyopsis*, '04, p. 206). In this manner also he would account for the presence in developing *Squalus* of a definite Reissner's fibre, which, he says, "has been traced forward under the Schaltstück" in embryos in which the "Dachkern" has not yet developed.

I shall show subsequently, when describing the development of the fibre in embryo *Amblystoma*, that the early stages of the development of the fibre are to be seen when neither habenular ganglia nor "Dachkern" are distinguishable. In these early embryos, however, the elements of the sub-commissural organ are already well defined, and it is from the ependymal epithelial cells of this sub-commissural organ that the numerous fine fibrillæ, which join up to constitute the fibre, have their origin, all of these fibrillæ arising posterior to the developing epiphysis, and remote, therefore, from the habenular ganglia. I shall also point out that the fibre arises far forward beneath the posterior commissure not only in *Amblystoma* but in all vertebrates, amongst which, in no single instance, do I find the fibre arising either wholly or in part from any point morphologically posterior to the posterior commissure, notwithstanding that in many forms (*Amphibia*, reptiles and birds) a well-developed "Dachkern" is present, which should

contribute a considerable branch if Sargent's account were correct.

Sargent devotes some space ('01, '04) to the consideration of the physiology of the "optic reflex apparatus," and gives an account of certain experiments performed by him upon living sharks. He says ('04, p. 231) that "these experiments, though incomplete, show clearly, I believe, that when Reissner's fibre is severed the power to respond quickly to optical stimuli is lost," which, even if it were established, is not at all the same thing as proof that the slowness of response said to have been observed is due either wholly or partly to the retardation of an optical stimulus.

He goes on to compute ('04, p. 240), from theoretical considerations, that the saving of time effected in the transmission of an optical stimulus along the "short-circuit" path afforded by Reissner's fibre would probably amount to at least  $0.016 + x$  seconds (where  $x$  is the delay in one cell-body), or approximately a saving of less than one fiftieth of a second, which is surely an insignificant result for such an elaborate and special apparatus.

In his earlier paper ('01) Sargent had denied the existence of the fibre in blind animals, and stated (*op. cit.*, p. 451) that "experiments are now in progress to determine the effect of artificial extirpation of the eye on this apparatus." The value of such experiments is obvious if, as Sargent believed, the fibre were absent in blind animals, and it would be of considerable interest to know what results were obtained, but in none of Sargent's later works is there any further reference to these experiments, and, as shown above, Sargent had subsequently to admit the existence of a fibre of Reissner in some blind animals, although, as already noted, he endeavoured to explain it away as being probably a case of the fibre functioning solely as an olfactory reflex apparatus.

In discussing the work of Houser, or rather that part of it which relates to Reissner's fibre, it is necessary first to consider to what extent his conclusions may have been influenced



by those of Sargent's works which had at that time been recently published. It must accordingly be borne in mind that in April, 1901, when the second of Sargent's preliminary papers appeared, that author had not apparently attempted to retrieve the mistakes consequent on his initial errors of identification. It is clear, too, from Sargent's descriptions ('01, p. 447-448) and figure (pl. 2, fig. 9) of the condition of Reissner's fibre in Elasmobranchs (as studied by him in very young *Raia erinacea*), that he had not at that time discovered how very far forward the fibre really extends beneath the posterior commissure, which structure, indeed, with its related sub-commissural organ (ependymal groove), he did not, even then, so much as mention.

Into practically identical errors both of omission and of commission, Houser, as I shall proceed to show, also fell. Indeed, it would appear that upon this subject Houser has blindly followed the first lead given by Sargent, while Sargent himself, when giving in his last paper ('04) an account that differs essentially in certain important details from his earlier accounts, yet claims ('04, p. 164) that "Houser . . . has fully confirmed my results as set forth in my preliminary papers"!

Although describing, in Reissner's fibre and its alleged cellular connections in the brain, a structure hitherto undescribed in adult Selachians, Houser failed to give any illustration which would enable his readers to gather clearly at precisely what point "the well-marked tract" of fibres "emerges from the mid-brain roof to penetrate the aqueduct, of Sylvius as the fibre of Reissner" (op. cit., p. 130). Indeed, in none of his numerous figures does he represent Reissner's fibre at all, and the absence, in particular, of any illustration showing precisely where he believes Reissner's fibre to emerge from the mid-brain substance into the aqueduct of Sylvius is the more to be regretted on account of the vagueness of his descriptions.

As is well known, the posterior commissure is included by many authors (Edinger, '08, Johnston, '07) in the mid-brain,

while by others (Burekhardt, '95, Studnička, '05) it is considered as part of the dience-phalon, and as, so far as I can find, Houser nowhere even mentions the posterior commissure, I am totally unable to decide what exactly that author regards as the "anterior limit of the mid-brain," but it seems probable that in this particular his account was intended to agree closely with the account given by Sargent of the origin of the fibre in Raia. The latter author had represented the fibre as emerging wholly behind the posterior commissure.

Of the cells of the "Dachkern," whose axons were supposed to unite to form Reissner's fibre, Sargent had said ('01, p. 447): "The cells are multipolar, giving off several processes in addition to the large axon, which is  $2\mu$  to  $3\mu$  in diameter," and "The axons pass dorsad and laterad from their cells, turning either cephalad or caudad" (my spaced type). Houser, more explicit, stated (op. cit., p. 129) that the axons from those of the cells in the anterior region of the tectum opticum pass cephalad to form Reissner's fibre, while those from the more posterior cells run caudad into the cerebellum. Neither he nor Sargent explained how such a very large number of these axons (in young Raia, according to Sargent, there are three or four hundred "Dachkern" cells with axons from two to three micra in diameter) could possibly become compressed into Reissner's fibre, which has, according to the later account of Sargent ('04, p. 147), even in the adult only a diameter of 6.7 micra. Sargent, however, at a subsequent period, discriminated between finer axons running cephalad to constitute Reissner's fibre and coarse neurites passing posteriorly to the cerebellum, and, ignoring his own earlier statements, dissented from Houser's account, remarking ('04, p. 177): "I believe that each cell sends an axon anteriorly, and also a cerebellar neurite posteriorly."

It is clear, moreover, from figures in Sargent's later work ('04) that he admits the existence in Raia, Mustelus and other Selachians of a relatively considerable part of Reissner's fibre (the anterior branch) unrelated to the "Dachkern." This runs forwards well beyond the point where the alleged fibre-tracts



were supposed to emerge. These latter, therefore (which had constituted the whole of the fibre in *Raia* as originally described by Sargent, and in *Mustelus* according to Houser), could form but a fraction of the entire fibre, so that the difficulty of accommodating these several fibre-tracts within the compass of a single thread whose diameter is little more than that of a coarse nerve-fibre is increased rather than diminished.

The condition of the adult *Mustelus* described by Sargent ('04) thus differs essentially from the condition that this author had previously ('01) figured and described for *Raia*, yet Houser, in the meanwhile ('01), had claimed to have found the fibre in the adult *Mustelus* in precisely that condition which Sargent had described for *Raia*.

Again, Sargent records that "the exact method by which the fascicles enter the ventricle and form Reissner's fibre has been difficult to make out in *Raia*, the connections having been broken away in all my series of sections" ('04, p. 169). He further admits ('04, p. 173) that in *Mustelus*, too, "near the upper limit of the ependymal groove the fibres are lost, but apparently they pass between the ependymal cells into the groove," and "a direct connection between the fibre-tracts described and Reissner's fibre has not been observed in this species." Thus it would appear that Sargent never actually saw in Elasmobranchs that emergence of the constituent axons "between the ependymal cells" which both he and Houser so confidently describe. That Houser, likewise, did not see this "emergence" of these "fibre-tracts" is probable, for in this connection it is not without significance that Houser altogether omits (just as Sargent before him had done) all mention of the posterior commissure, and entirely overlooks the sub-commissural organ (ependymal groove), to which Sargent had not at that time directed attention, although the latter author afterwards described this structure and considered it to be developed as an anchorage and support for Reissner's fibre. This oversight on the part of Houser is the more astounding seeing that he devotes a considerable part

of his paper to an account of the supporting elements (ependyma and neuroglia) in the various regions of the brain, and that it happens that the sub-commissural organ reaches nearly, if not quite, its maximum development in Selachians (see Text-figs. 2, A, B, and 3, A, and figs. 2, 3). That any observer, especially one professing to make a study of supporting elements, could have seen the emergence of the constituent "axons" or "fibre-tracts" (so-called) of Reissner's fibre into the mesocoel, and yet have failed to notice this extraordinarily developed ependymal epithelium is almost incredible.<sup>1</sup>

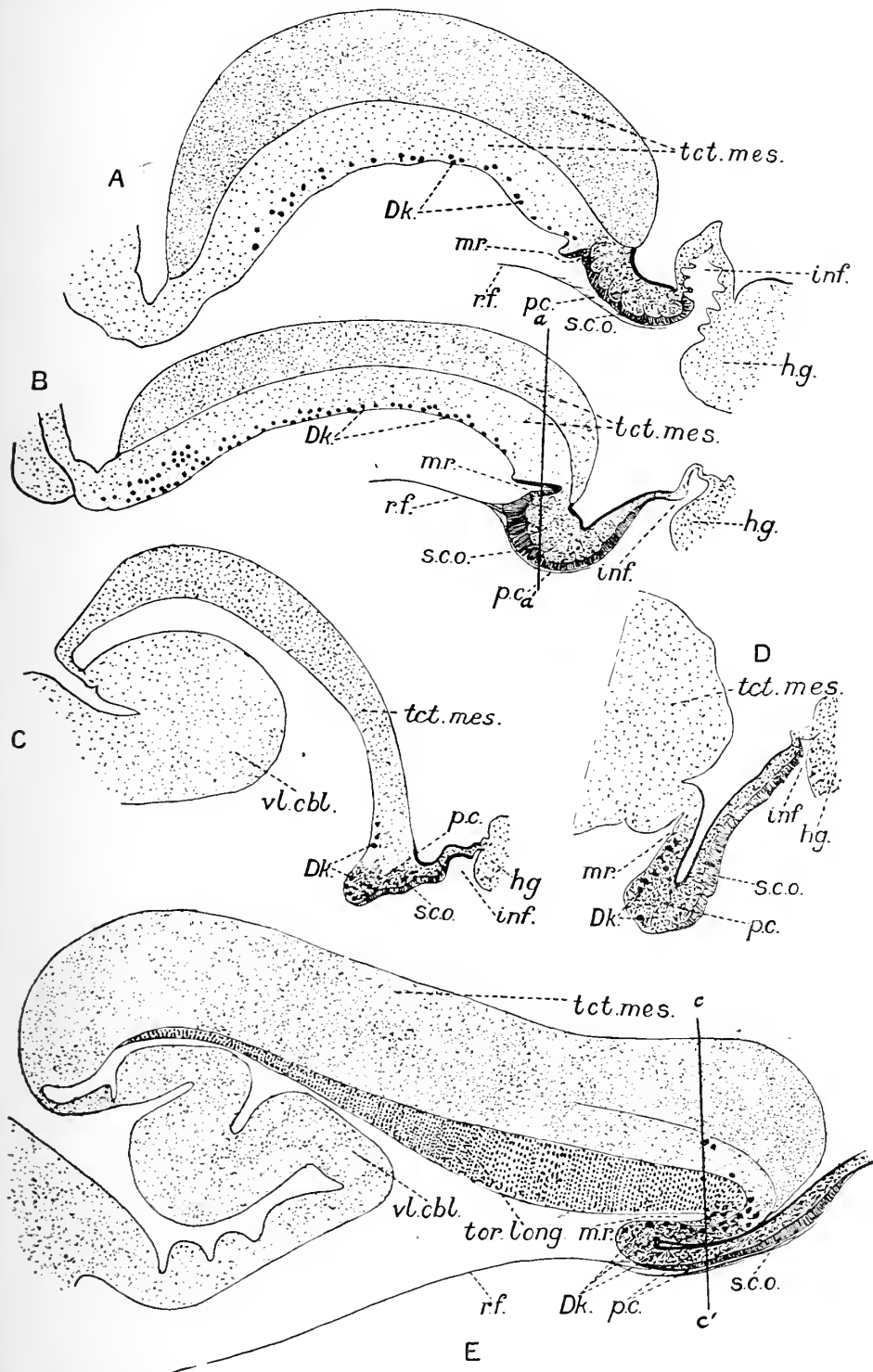
Concerning the "Dachkern" Houser remarks: "It has remained for Sargent (1900) to show that not only is the roof nucleus present in all vertebrates but that it is a part of a most interesting mechanism, the fibre of Reissner" ('01, p. 132). This statement is by no means correct, for although the existence of the cells of this "roof nucleus" had been described in some cases long before in several classes of vertebrates, yet, so far as I can find, this nucleus has never been identified in mammals, nor at that time had it been identified in Teleosts. Subsequently Sargent stated that the *torus longitudinalis* was the homologue of

<sup>1</sup> Studnička ('00), in the previous year, had described and figured this modified ependymal epithelium in *Scyllium*, remarking that he was not able to determine its function, while, so far back as 1892, Edinger ('92) had called attention to it, suggesting that it might have some secretory function.

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TEXT-FIG. 2.—Slightly diagrammatic median sagittal sections through the mid-brains of (A) *Raia blanda*; (B) *Scyllium canicula*; (C) *Polypterus* sp.; (D) *Amia calva*; (E) *Esox lucius*; to show the apparent shifting of the position of the "Dachkern" in fishes. [(D) Has been borrowed from Sargent's ('04) paper, while in (C) the outline has been taken from a figure by Graham Kerr into which the "Dachkern" cells have been drawn from the figure by Sargent ('04, Pl. VI, fig. 41).] *Dk.*, "Dachkern." *h.g.*, Habenular ganglion. *inf.*, Infra-pineal recess. *m.r.*, Mesocoelic recess. *p.c.*, Posterior commissure. *r.f.*, Reissner's fibre. *s.c.o.*, Sub-commissural organ. *tect. mes.*, Tectum mesencephali. *tor. long.*, Torus longitudinalis. *vl. cbm.*, Valvula cerebelli. (The lines *aa'* and *cc'* indicate approximately the levels at which the sections represented in Text-fig. 3, A, C, were taken.)

TEXT-FIG. 2.

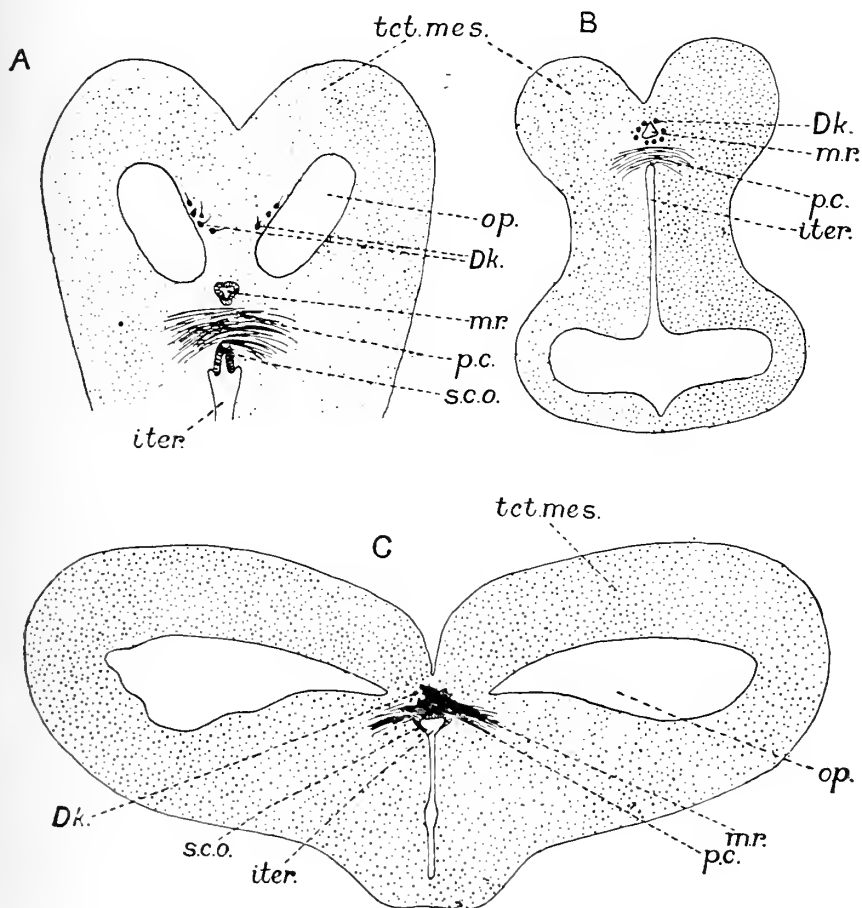


the "Dachkern" of other vertebrates. This suggested homology, as already indicated, I am quite unable to accept, for I shall show that the "Dachkern" is present in a scarcely modified condition in some primitive teleosts in which the torus is also present as a well-developed structure.

That the "Dachkern" has, so far as I can find, never hitherto been recognised in the Teleostean brain is doubtless principally due to the fact that it has apparently shifted its position, and in many Teleosts is greatly reduced in importance, or may even have become obsolete. This apparent change of position is the result of the immense development, in Teleosts, of the tectum mesencephali (the anterior border of which is fixed at the posterior commissure), so that that part of the tectum originally anterior becomes inrolled to form the floor and part of the front wall of the optocœl, where that cavity extends forward above the posterior commissure. It retains, in some Teleosts at any rate, the condition described by Sargent ('04, pp. 188-189, fig. D) for adult Ganoids, and it is remarkable that Sargent, who realised, and, indeed, laid some stress upon the forward shifting of the fore part of the mid-brain, should have overlooked the group of conspicuous cells which occupies in Teleosts a position practically identical with that of the "Dachkern" in Ganoids, and strictly comparable with that occupied by this nucleus in other classes of vertebrates. This question will be more fully discussed when we come to speak of the Teleosts, but in the meantime the above statement may be rendered more intelligible by reference to Text-figs. 2 and 3.

From what has been said it is evident that Houser's observations appear to be quite in agreement with those which Sargent announced in his preliminary papers. In those papers, however, the optic reflex theory had been stated in the barest outline only, and Houser, readily accepting the theory, proceeded to explain and amplify it. In the result, his presentation of the facts and theory

TEXT-FIG. 3.



Slightly diagrammatic transverse sections through the anterior portion of the mid-brain of (A) *Scyllium canicula*; (B) *Acipenser* sp.; (C) *Esox lucius*; to show the relations of the cells of the "Dachkern" to the Tectum mesencephali, Recessus mesocœlicus, and posterior commissure. [(B) is borrowed from Johnston's work on the brain of *Acipenser* ('01). A and C are taken at levels indicated by the lines *aa'* and *cc'* in Text-fig. 2, A and E.] *Dk.* "Dachkern." *iter.* Iter. *m.r.* Mesocœlic recess. *op.* Optocœl. *p.c.* Posterior commissure. *s.c.o.* Sub-commissural organ. *tct. mes.* Tectum mesencephali.

prove to be almost entirely different from the account and explanation subsequently offered by Sargent.

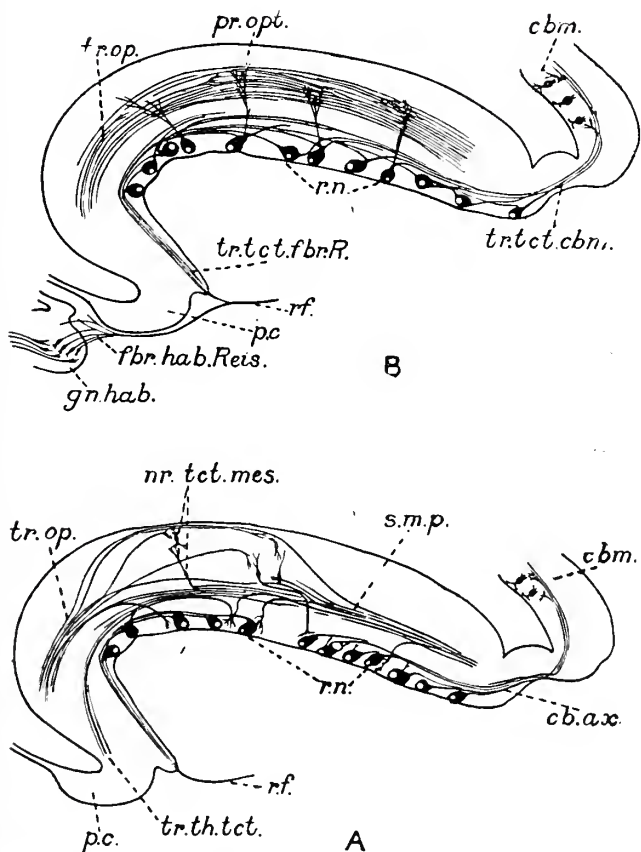
In order that these different accounts may be more readily compared I have, in Text-fig. 4, endeavoured to represent quite diagrammatically what is alleged by these two authors concerning the cellular connections of Reissner's fibre.

As I understand it, Sargent's view (Text-fig. 4, B) supposes that sensory stimuli (optical and olfactory) reach certain cells in the tectum mesencephali (those of the torus in Teleosts, and of the "Dachkern" in other vertebrates), and in the habenular ganglia. These "Dachkern" and torus cells are said to be multipolar, each being stated to give off (i) an "axon," which courses anteriorly to join Reissner's fibre; (ii) a "neurite," ultimately passing posteriorly into the cerebellum, there to "end in fibrillations in the molecular layer" in direct contact with the processes of the Purkinje cells; and (iii) a process which makes its way "towards the ectal region of the tectum opticum," to come "directly in contact with the endings of the proximally running fibres of the optic nerve. It is by this process that the cell is put in direct connection with the outer world by the retina" ('04, p. 168)—the spaced type is mine. The "Dachkern" cells are clearly regarded as motor, for Sargent says that their axons "pass by the shortest route through the ventricle and canal to the posterior portion of the nervous system, where they pass into the cord and probably pass out through the ventral root to the musculature" ('01, p. 450). The habenular cells (which in Cyclostomes, at any rate, are said to be multipolar) also contribute axons to Reissner's fibre, which thus serves "as a short circuit for the transmission of reflexes arising from olfactory as well as optic stimuli" ('04, p. 162).

Sargent also claims that the fibre growing backwards from this cephalic part of the apparatus fuses with "axons" extending forwards from (motor?) cells lying wholly within the sinus terminalis, but nothing is said as to the anterior ending of these latter axons.



TEXT-FIG. 4.



Diagrams representing the cellular connections of Reissner's fibre in the brain, A, according to Houser ('01); B, according to Sargent ('04). *cb.ax.* Cerebellar axons of the "Dachkern" cells. *cbm.* Cerebellum. *fbr.hab.Reis.* Constituent axons of Reissner's fibre derived from cells in the habenular ganglia. *gn.hab.* Ganglion habenulae. *nr.tect.mes.* Neurons of the Tectum mesencephali. *p.c.* Posterior commissure. *pr.opt.* Processes (dendrites) of the "Dachkern" cells ending amongst the fibres of the Tractus opticus. *rf.* Reissner's fibre. *r.n.* Cells of the "Dachkern" or roof nucleus. *s.m.p.* Stratum medullare profundum. *tr.op.* Tractus opticus. *tr.th.tet.* Tractus thalamo-tectalis. *tr.tet.cb.* Tractus tectalis cerebellaris. *tr.tet.fbr.R.* Tractus tectalis fibrae Reissneris.

The very delicate strands often to be seen in the *canalis centralis*, that seem to connect the fibre of Reissner with the ependymal epithelium, are evidently the "axons" which are supposed to pass out with the ventral roots of the spinal nerves to the musculature ('04, pp. 188, 195).

According to Houser (Text-fig. 4, A), the *stratum medullare profundum*, which immediately overlies the "*Dachkern*" is a fibre system into which he claims to have "traced fibres from the cord and oblongata, fibres from the optic nerve, from the neurones of the *tectum mesencephali* itself. . . . a great tract sweeps into it from the interbrain as a relay in the olfactory apparatus. Fibres are also present from certain of the cranial nerves. All of these fibre systems are to become related to the remarkable mechanism of Reissner's fibre" ('01, p. 125).

Houser thus suggests the transmission along the short cut provided by Reissner's fibre of motor impulses consequent on sensory stimuli other than optic and olfactory, but he indicates paths quite different from those described by Sargent by which the sensory stimuli reach the cells of the "*Dachkern*." For although he concludes—"It is certainly evident that there are here every means for inter-communication between different parts of the nervous system," yet he traces a connection between this *stratum medullare profundum* and the cells of the "*Dachkern*," not as Sargent does, but by the passing of some of the fibres from the *stratum medullare profundum* itself down to the "*Dachkern*" there to end in arborisations over those cells.

It will thus be evident that the two explanations of the working of this optic reflex apparatus have very little in common, and it is, indeed, rather surprising to find Sargent citing Houser as confirming his results.

Moreover, I am quite unable to discover upon what grounds either Sargent or Houser have assumed the motor character of these "*Dachkern*" cells, for there appears to be absolutely no warrant for the assumption.

It is my own opinion that these large cells forming the "roof



nuc lens" will prove to be sensory, and to be simply a part of that series of dorsally placed giant-cells which are such a conspicuous feature in the central nervous system of Cyclostomes and fishes. In support of this view I shall point out that, in *Petromyzon fluviatilis*, the giant cells of the cord do not cease, as stated by Johnston for *P. (Lampetra) wilderi*, behind the commissura infima ('02, p. 5), but extend forwards almost to the cerebellum high up on the medulla on either side of the fourth ventricle. These in many instances project almost through the ependymal epithelium into the ventricle, exactly as the cells of the "Dachkern" commonly project into the mesocoel.

I find, also, a striking similarity in the staining reactions of the cells of the "Dachkern" and of the giant-cells of the cord in those forms where both are developed. Various observers, too, have noticed the failure either of the cells of the "Dachkern" or of the giant-cells of the cord to impregnate with Golgi's method. Further, Sargent remarks that (in young *Raia*) many of the tectal reflex cells are to be seen "apparently undergoing atrophy and degeneration, showing all the stages in the process that have been observed in the atrophy of the dorsal giant-cells of the spinal cord" ('04, p. 166).

In Elasmobranchs, amongst others, the dorsal giant-cells of the spinal cord have been said to completely disappear during embryonic life (Beard, '89, Studnička, '95B), whereas, as I shall describe in a subsequent paper, some, in the hinder part of the spinal cord, are to be found persistent in specimens of *Raia* six to eight inches long, and in adult dogfish. Not only so, but in the latter at any rate they grow to an enormous size and become multinucleate. It is, however, not without significance that some of the cells of the "Dachkern" should be undergoing marked degenerative changes so precisely similar to, and at the same period in development as, many of the giant-cells of the cord.

It is, I believe, now generally accepted that these giant-cells of the cord are sensory, and are derived from neuroblasts

of the neural crests. These neural crests are known to extend forward in development to the front end of the mesencephalon, being interrupted, however, in the region of the ear. The extent of the neural crests, therefore, coincides strictly with the regions in which occur the giant-cells of the spinal cord and the cells of the "Dachkern."

Thus, in their dorsal position adjacent to the cavity of the neural tube, in their general appearance, in their great size, in their possession of one particularly conspicuous non-medullated fibre, in their peculiar staining reactions and in their tendency to atrophy at the end of embryonic life, the cells of the "Dachkern" show a striking resemblance to the giant-cells of the spinal cord.

I have already called attention ('09, '12) to the remarkable elasticity of Reissner's fibre and to its behaviour in recoil in a manner quite unknown among nerve-fibres. Horsley has pointed out that in its failure to show degenerative changes after section it appears quite unlike a nerve-fibre, and this the results of my own experiments, some of which are already recorded ('12), entirely confirm. In its staining reactions, too, the fibre is altogether distinctive.

After continued investigation into the structure and mode of occurrence of Reissner's fibre in all classes of vertebrates, I find myself almost completely at variance with the views expressed by Sargent, and I repeat here the assertion that the fibre is not a nerve-tract. It arises from the specialised epithelium of the sub-commissural organ, which has markedly the character of a sensory epithelium. It is first discernible at a point far forward beneath (anterior to) the posterior commissure, where it is formed by the coalescence of numerous fine fibrillæ, resembling long cilia, from the elongated ependymal cells; these fibrillæ continue to join it along its whole course beneath the posterior commissure (and probably also in the *canalis centralis*); but it never arises, either wholly or in part, from any point in the brain dorsal (posterior) to the posterior commissure in any form which I have studied, except, perhaps, in a comparatively few cases

(e.g. Selachians and Myxinoids), where the specialised ependymal epithelium also may extend onto the dorsal (posterior) surface of the posterior commissure.

### III. REISSNER'S FIBRE AND THE SUB-COMMISSURAL ORGAN IN THE PETROMYZONTIDÆ.

#### *Petromyzon fluviatilis.*

Of this species I have specially prepared and studied seven series of sections of the brain cut in the usual three planes, while of the terminal part of the spinal cord I have five series cut sagittally. In addition to these I have also examined several series through the brain and through the tail region of this animal belonging to the collection of King's College.

Upon the brain of one and another of the several species of this family so much has been written (Ahlborn, '83, on *P. planeri*; Dendy, '02 and '07, on *Geotria australis*; Johnston, '02, on *Lampetra wilderi*; Sargent, '04 and Sterzi, '07, on *Petromyzon marinus*, to mention only a few of the many modern writers who have dealt with the region of the brain with which I am here principally concerned), that there will be need for me to say very little concerning the gross anatomy of the brain of *Petromyzon fluviatilis*.

#### Sub-commissural Organ.

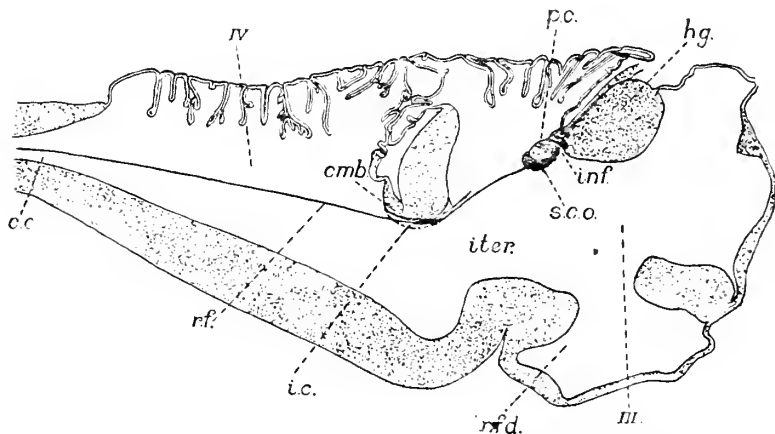
Although a very conspicuous structure, the sub-commissural organ has apparently been described in some detail in three species only, viz.: *Ammocætes* (*Petromyzon*) sp. (Dendy, '02), *Geotria australis* (Dendy, '02, '07), and *Petromyzon marinus* (Sargent, '04).<sup>1</sup> In these, it is

<sup>1</sup> Johnston ('07) does not even mention this structure, and Sterzi merely speaks of a highly developed epithelium lining the "recessi post-abenulari" in *P. marinus*. This he supposes (as Edinger before him had done in the case of *Scyllium*) to be a secretory epithelium.

stated to take the form of paired bands of high columnar ependymal epithelial cells, which are grouped so as to bound more or less deep longitudinal grooves, arising anteriorly immediately behind the habenular ganglia and extending backward beneath the posterior commissure.

In *Petromyzon fluviatilis* I find a similar disposition of this characteristic epithelium. It appears anteriorly as a well-defined band in the roof of each of the narrow clefts which separate on either side the downwardly projecting

TEXT-FIG.



A slightly diagrammatic median (sagittal) section through the brain of *Petromyzon fluviatilis*. One of the two factors of Reissner's fibre is shown from the sub-commissural organ backwards, the other branch is indicated near their junction at the anterior end of the isthmus canal. (In a truly sagittal section neither would be seen in front of that point in the mid-brain, for they actually lie just to the right and left of the median plane.) *c.c.* Canalis centralis. *cmb.* Cerebellum. *hg.* Habenular ganglion. *i.c.* Isthmic canal. *inf.* Infra-pineal recess. *inf.d.* Infundibulum. *iter.* Iter. *p.c.* Posterior commissure. *rf.* Reissner's fibre. *s.c.o.* Sub-commissural organ. *III.* Third ventricle. *IV.* Fourth ventricle.

habenular ganglia from the side walls of the diencephalon (Fig. 33, *s.c.o.*). Owing to the greater size of the right habenular ganglion the cleft upon that side is almost obliterated and the corresponding ependymal groove crowded out.

It extends forward, therefore, for a much shorter distance than does that of the opposite side, a condition which Dendy ('02, p. 489) noted in the ammocete of *Petromyzon* and, later, in the velasia stage of *Geotria* ('07, p. 5). Sargent states that he found in *Petromyzon marinus* the right groove better developed anteriorly, "corresponding in this with the greater size of the habenula of that side" ('04, p. 152).

Immediately behind the habenular ganglia these lateral clefts (in the roofs of which lie the forward extensions of the sub-commissural organ) widen out into and become merged with the infra-pineal recess. The bands of columnar epithelium also expand, spreading out upon the roof and side walls of that recess (fig. 34). The upper limit of the infra-pineal recess lies at a slightly higher level than the dorsal surface of the posterior commissure, and at that level there are always found a pair of shallow pockets or "diacœlic recesses" bulging very slightly laterally and backward, thus appearing above the posterior commissure. Owing to the spreading out of the paired epithelial bands of the sub-commissural organ upon the roof and side walls of the infra-pineal recess almost all of the upper region of the recess comes to be lined by this epithelium, and the pair of diacœlic recesses are completely lined by it. Being paired laterally placed structures they do not appear in the diagrammatic representation (Text-fig. 5) of a truly sagittal section, but the recess<sup>1</sup> of the right side is shown in fig. 35 (*r.d.*, on the left in the figure), only one being shown on account of the slight obliquity of the plane of the section. From the infra-pineal recess the paired tracts of specialised ependymal epithelium extend downward and backward beneath the posterior commissure, sharply marked off from the general ependymal epithelium. They bound distinct and, for the most part, widely separate grooves,

<sup>1</sup> These recesses appear to be identical with the "recessi postabenu-lari" described by Sterzi ('07) in *P. marinus*. These latter are apparently much larger than the diacœlic recesses I have described in *P. fluviatilis*, and Sterzi seems to have overlooked them in the latter species.

whose lumina face ventro-mesially (figs. 1, 35, 38, *s.c.o.*). This species thus differs from both *Petromyzon marinus* and *Geotria australis*, in which the lumina of the grooves face directly ventrally. It differs, also, from *Geotria* in that, in the latter, the two ependymal bands are in contact mesially.

The epithelium of the sub-commissural organ is composed of elongated, almost fibre-like cells, radially arranged and with deeply seated nuclei which stain very strongly. The cytoplasm of these cells usually stains comparatively lightly, so that, in transverse sections, the sub-commissural organ appears as a pair of pale crescentic areas whose outer bounding curves are defined by closely set nuclei (figs. 38, 39). The inner ends of the cells of the sub-commissural organ are produced into long neuroglia fibres which become collected into bundles and extend to the dorsal surface of the brain, the nerve-fibres of the posterior commissure passing between them.

Laterally the sub-commissural organ is sharply marked off from the general ependymal epithelium, which consists of short columnar cells with more superficially disposed nuclei (fig. 38, *e.ep.*). This general ventricular ependymal epithelium seems to be freely furnished with long cilia, but these are replaced upon the sub-commissural organ by very short, close-set cilia. The latter are not always preserved, but are occasionally quite conspicuous, especially upon that part of the sub-commissural organ which lies in the infra-pineal recess.

Mesially, beneath the posterior commissure, those cells which form the dorsal edges of the grooves are much less elongated, and finally there is a transition into an epithelium of flattened cells which alone cover the ventricular surface of the posterior commissure between the two halves of the sub-commissural organ (fig. 38). This flattened epithelium continues around the hinder end of the posterior commissure and forms the only epithelial covering of the dorsal (posterior) surface of that structure, for onto this surface the sub-commissural organ does not extend in *Petromyzon fluvialis*. Thus, at the hinder end of the posterior commissure



the sub-commissural organ ends abruptly (see Text-fig. 5). Owing, however, to the great length of their component cells the two halves of the sub-commissural organ continue to appear for a short distance in transverse sections as crescentic epithelial structures, lying almost or quite freely in the mesocœl altogether behind the commissure (fig. 36, *s. c. o.*).

This manner of ending of the sub-commissural organ is absolutely characteristic of all the members of the Petromyzontidæ which I have examined. In *P. marinus*, however, Sargent states ('04, p. 152) that the sub-commissural organ ("ependymal grooves") extends around the postero-ventral surface of the posterior commissure onto the dorsal (posterior) surface of that structure, although his figures ('04, pl. i, figs. 6 and 7) do not seem to bear out his statement. I shall return to this matter later.

### Reissner's Fibre.

Springing from the epithelium of the sub-commissural organ, where this lines the infra-pineal recess on either side, a number of delicate fibrillæ may be made out which coalesce to form factors of Reissner's fibre (fig. 39, *fb.*). Mingled with these fibrillæ are numerous fine strands, which I was at first inclined to consider as artifacts due to coagulation. They are, however, invariably present at this point, and closely resemble the constituent fibrillæ of Reissner's fibre, differing principally in that they have a slightly irregular and wavy course, whereas the undoubted constituents of the fibre are straight, as though drawn taut under considerable tension. It may well be that the loose wavy fibrillæ (fig. 39, *fb.*) are simply factors of Reissner's fibre which have been broken and contracted under the action of the fixing reagent.

The sub-commissural organ, as already stated, consists of separate paired bands, which in *Petromyzon fluviatilis* nowhere coalesce nor even closely approach, and it is an interesting fact that in this species the fibrillæ unite to form a pair of principal factors (fig. 37, *r. f.*) of Reissner's fibre.

These remain distinct for a considerable distance through the brain cavity, uniting, in a manner shortly to be described (fig. 11, *r.f.*), to form a single median structure only beneath the posterior part of the tectum mesencephali, immediately in front of the point where that structure joins the cerebellum (Text-fig. 5).

The anterior surface of the posterior commissure, over which the paired ependymal grooves pass, has a presentation approaching the vertical (see Text-fig. 5), but slopes slightly backwards. The course of the paired fibres, therefore, is at first steeply downward and slightly backward. Moreover, they also incline somewhat towards the middle line, and it is thus not possible (in this species) to obtain any considerable length of the fibre in this region in any one (thin) section cut in any of the three conventional planes.

In transverse sections especially the fibre is, in this region, cut very obliquely, and, as it lies in this part of its course closely against the ependymal epithelium, it frequently may, by focussing, be seen apparently penetrating this epithelium. Really, of course, it simply lies against the ependymal epithelium upon its free surface (fig. 38, *r.f.*).

Lying close adjacent to the ventral surface of the posterior commissure, and separated from the aqueductus Sylvii only by that flattened epithelium which extends mesially between the two grooves of the sub-commissural organ, are numerous conspicuous nerve-fibres. These are the axons of the large, laterally situated cells (fig. 36, *n. p. c.*), which Johnston ('02) has identified as the nucleus of the posterior commissure, and they are the nerve-fibres which form, according to Sargent ('04, pp. 154-155, pl. i, figs. 6 and 7), the second of the three sources from which he would derive Reissner's fibre in the lamprey. Owing to the extremely flattened character of the ependymal epithelium here, many of these axons lie quite superficially. Where they pass laterally into the region of the sub-commissural organ they still continue, for a while, to course superficially between the radiating fibre-like ependymal cells, presenting occasion-



ally the appearance of passing out towards the iter. This appearance is to be observed more frequently in transverse sections through the anterior parts of the posterior commissure, where the sections cut its antero-ventral surface very obliquely.

Such a condition is represented in fig. 38, where all the more conspicuous fibres have been carefully drawn in, with the aid of a camera lucida, from an actual section which formed part of a complete series stained with iron-brazilin. As one would naturally expect, these fibres could be followed from section to section, past the point where they might have appeared to emerge, to the side of the posterior commissure remote from that upon which their related cells lay.

It was, perhaps, his failure to interpret correctly appearances such as these that led Sargent to suppose that he could trace axons into the ventricle; while the obliquely cut sections of Reissner's fibre, to which I have already referred as seeming to penetrate the ependymal epithelium, may have been regarded as the continuations of such axons.

The utter improbability of such an origin for the fibre of Reissner, from the confluence of a large number of neuraxons, will become apparent when it is stated that in many instances an individual fibre from this laterally placed group of cells has a diameter as great as, or even greater than, that of the entire Reissner's fibre itself, while collectively (and they are quite numerous) they would vastly exceed that structure in size.

Furthermore, according to Sargent, these constituent fibres form but a fraction of the entire fibre in *Petromyzon*, for other "axons" are described as entering into it (1) from a paired nucleus of large cells, which he correctly homologises with the "Dachkern," and which lies dorsal or dorso-lateral to the posterior commissure, and (2) from large multipolar cells in the habenular ganglia.

As a matter of fact, the ultimate factors of Reissner's fibre are exceedingly fine, and can be traced only to the free surface of the elongated cells of the sub-commissural organ (fig. 39, *fb.*).

Beneath and behind the posterior commissure the right and left halves of the fibre pass backward and ventrally in a nearly parallel course, but converging slightly (fig. 37, *r.f.*), till, beneath the rhombo-mesencephalic fold, they may be seen, in a series of transverse sections, to enter a pair of deep grooves on the ventral surface of that fold (fig. 10, *i.c.*). These paired grooves after a short separate course become confluent behind to form a single median groove, which continues backward to the extreme caudal end of the ventral surface of the fold. This groove, which I propose to call the "isthmic canal" (Text-fig. 5, *i.c.*), is lined by a distinct columnar epithelium, which is much more strongly staining than the ependymal epithelium which covers the rest of the ventricular surface of the rhombo-mesencephalic fold. It is of very general occurrence, at any rate in the lower vertebrates, and probably increases in depth during life. In the Myxinoids, as will be described below, this isthmic canal becomes converted into a tubular structure.

At the point of confluence of the paired anterior parts of the isthmic canal the paired branches of Reissner's fibre also unite to form a single median thread, which lies in the groove close against the ependymal epithelium. In the photomicrograph reproduced as fig. 10, the pair of fibres (*r.f.*) may be seen in the two halves of the isthmic canal at a point about twenty micra in front of the confluence of the canals. In other series of transverse sections through the brain of *Petromyzon*, the isthmic canal does not show the paired anterior portion, and the two halves of the fibre appear to unite slightly antero-ventral to the rhombo-mesencephalic fold (fig. 11, *r.f.*). These are probably younger specimens, for this difference seems to be due to a difference of age and degree of down-growth of the brain-tissue upon the fibre. I have found a similar difference in the frog, where in a young specimen the fibre lies freely beneath the ventral surface of this part of the brain, while in an older (fully grown) specimen the fibre has come to lie in a deep isthmic canal.

Behind the cerebellum the fibre (Text-fig. 5, *r.f.*) emerges

from the isthmic canal and extends perfectly freely through the fourth ventricle, from which it passes into the *canalis centralis* to end in the *sinus terminalis*. In no part of its course does it penetrate the brain-tissue, and the isthmic canal, in all of the seven series examined, remains widely open below. In two series of sections the fibre had evidently broken from its attachment to the sub-commissural organ during the dissection made to expose the brain, and has sprung backward into the central canal of the spinal cord. In both cases, however, the process of fixation must have been well advanced, especially in the relatively less bulky spinal cord, and the fibre has consequently retracted only from the brain into the anterior region of the cord. In one case the recoil has merely resulted in a shortening up of the anterior part of the fibre and a considerable increase in its thickness in that region. In the second case the released anterior end of the fibre has been twisted into a spirally coiled mass of swollen fibre which lies at the point where the fourth ventricle passes into the *canalis centralis*. It is associated with a considerable quantity of dislodged ependymal cells, and on examination it was found that the sub-commissural organ is somewhat incomplete, as though patches of cells had been dragged away when the fibre tore itself free.

Throughout the extent of the *canalis centralis* the fibre is attached, at frequent intervals, to the ependymal epithelium by cilia, which appear to have fused with the fibre, and which probably are, as I have suggested above, actually constituent parts of Reissner's fibre.

I have been able to trace Reissner's fibre with absolute certainty to its actual end in the *sinus terminalis* in this species in but two specimens. This is due to the fact that the fibre can only be certainly followed in sagittal sections, the preparation of which, in this region, presents some difficulty. The spinal cord extends as an exceedingly delicate, tapering *filum terminale* to the extremity of the tail. It is supported ventrally by the notochord, beyond the posterior end of which, however, it projects slightly, there becoming

dilated to partly enclose the sinus terminalis. Above, its meningeal sheath is protected only by the skin, with which it lies intimately in contact. Removal of the skin appears invariably to involve the hinder part of the dural envelope of the spinal cord also, and, as a consequence, the sinus terminalis, of whose wall that envelope forms an integral part. If, however, the skin be not removed, the whole tail becomes greatly crumpled and folded during the processes of paraffin embedding, owing to the considerable shrinking of the skin, which itself becomes at the same time very tough and leathery. Thus, in those series in which the end of the spinal cord had been dissected out (specimens A, B) or in which the skin had simply been removed (specimen C), the sinus terminalis was wanting. On the other hand, in both of the two series of sections (specimens D, E) in which the intact tail region was cut sagittally, it came about that the sinus terminalis, although preserved entire, was unavoidably cut slightly obliquely to the vertical longitudinal plane desired.

A photomicrograph of a section through the sinus terminalis of each of these two latter (D, E) has been reproduced (figs. 12, 13, *s.t.*), while fig. 40 is a composite drawing obtained by superposing camera drawings of four or five consecutive sections of the series through the tail of specimen D.

The sinus (ventriculus) terminalis in *Petromyzon fluviatilis* may be seen in both of my complete series as a somewhat ovoidal space formed by the widening out of the hinder end of the canalis centralis. By Studnička ('95A) it is said to be invariably present in both *P. fluviatilis* and *P. planeri*; Retzius ('95) says simply that in *P. fluviatilis* it frequently occurs.

The anterior part of the wall of this space is formed by the ependymal epithelium of the filum terminale. At about its middle, however, this fails, so that posteriorly the sinus terminalis is bounded only by the confluent connective tissue envelopes of the spinal cord (Fig. 40). The canalis

centralis may thus be said to open freely by a terminal neural pore or foramen into the lymph-space that surrounds the cord. Through this terminal foramen Reissner's fibre passes, to become inserted into, and apparently confluent with, that portion of the meningeal sheath that forms the posterior wall of the sinus terminalis. Its insertion is in the middle line and upon the postero-ventral part of the wall of the sinus.

In fig. 13 the fibre is seen passing across the sinus terminalis to its point of insertion into the wall of that chamber, and in this instance the fibre shows only a slight twisting at short intervals. In the other specimen (figs. 12, 40), the fibre is seen to pass into the apex of a conical mass (*r.f.*) of coiled fibre which lies against the ventro-posterior wall of the sinus and occupies a considerable portion of its cavity.

The descriptions of Studnička ('99) and of Sargent ('04), as well as my own earlier experience (Nicholls, '09), had prepared me for a complete or partial recoil of the fibre in this region. In order to prevent this and to obtain, as far as might be, a representation of the actual condition in the living animal, considerable care was exercised in the preparation of the material. With this end in view, two freshly killed lampreys were taken and immersed whole in the fixing fluid (aceto-bichromate), and, while so immersed, a short stretch of the skin and muscles was removed from one side of the tail to within about an inch from its end to allow of the better and more speedy penetration of the spinal cord by the fixing fluid, without any risk of damage to, or displacement of, the spinal cord and enclosed Reissner's fibre. This last inch or so of the tail was only cut off nearly an hour later, by which time it was supposed that the fibre had become well fixed and its elasticity destroyed. The tissue was then further hardened in the fixing fluid for another twenty-four hours or more.

In the case of specimen E subsequent examination of the sections showed that there had been no post-mortem recoil of the fibre, which could be traced forward uninterruptedly from the sinus terminalis in a perfectly straight line through the canalis centralis. It is thus practically certain that the

slight knotting and coiling to be observed in the fibre (fig. 13) must have existed in life. In specimen D, however, the cord and Reissner's fibre were clearly severed prematurely, before the latter had been quite fixed at its posterior end. A certain amount of recoil had taken place, so that for about three millimetres the canalis immediately behind the point of cutting had had the fibre withdrawn from it. Where the fibre appears it is found to stretch back in a tolerably straight piece almost to the sinus terminalis. About at the point, however, where the central canal begins to widen out, just anterior to the sinus terminalis, the fibre passes into a considerable tangle (fig. 40, *r.f.'*), from the hinder end of which it emerges to run in a short straight course into another and much larger tangled mass (fig. 40, *r.f.''*). This second tangle is that conical heap which forms so conspicuous an object in the sinus terminalis (fig. 12, *r.f.''*), the base of the cone lying against the postero-ventral wall of the sinus terminalis. Its apex, into which the straight part of the fibre passes, projects dorsally and a little forwards. As a post-mortem recoil has here undoubtedly occurred, it is not now possible to decide whether any of this tangled mass of the fibre was present as such in the living animal. It is my opinion, however, that the intricately tangled mass (fig. 40, *r.f.'*) lying near the end of the central canal is alone sufficient to account entirely for the comparatively small amount of retraction from the anterior end to which I have referred.

In addition to this material which I myself prepared I have examined a number of series of sections through the tail of *Petromyzon fluviatilis* which are in the collection at King's College. In two of these I found the sinus terminalis almost intact, but in one only could Reissner's fibre be traced backwards to that point. In that series, which was cut horizontally, the fibre may be seen emerging from a widely open terminal neural pore as a taut thread, which passes at its extremity into a mass of indeterminate tissue and is lost. The meningeal walls of the sinus terminalis in this specimen are very ill-defined.



In the other series, a sagittal one, the sinus terminalis was cut obliquely. It appeared, however, to be a well-defined globular space. The anterior hemisphere was covered with the spreading flattened ependymal epithelium of the filum terminale, but this was lacking on the hinder half, and here the wall was made up wholly of connective tissue. The sinus itself was filled with a granular coagulum and Reissner's fibre could not be identified.

The effect obtained by a severance of cord and fibre when fixation is still incomplete has already been figured and briefly described by me ('09, '12). The evenly twisted condition of Reissner's fibre, to which I have referred as occurring in those cases where only a gradual recoil of the fibre has taken place, has been observed in the central canal of three different specimens of *Petromyzon fluviatilis*.

The first case of this kind was obtained in the terminal portion of the spinal cord, which had been dissected out from the vertebral canal after partial fixation. The cord and fibre were cut in front and retraction of the fibre has taken place backwardly from that point. In the operation of dissecting out the piece of spinal cord, however, the sinus terminalis was destroyed and the fibre has also retracted forwards, but it appears probable that the retraction took place principally from before backwards, for from the point where the fibre was cut the canalis centralis is empty of fibre for a space of nearly fourteen millimetres. The anterior part of the piece of fibre in question is almost perfectly straight and not greatly swollen. This straight course continues for nearly a millimetre and then the fibre appears thrown into a number of close, tightly wound, corkscrew-like coils which alternate with short straight stretches (fig. 16). Of these corkscrew-like coils some thirty occur in the hinder part of the fibre, which is altogether about three millimetres only in length. The number of turns in any one twisted length varies from four to fourteen, with an average of eight. Occasionally, however, a single turn is found in an otherwise straight piece.



In addition to the coiling there has been an actual shrinking in length and a corresponding increase in thickness, for the fibre, which has normally in the lamprey a diameter of 1.5-2 micra, has here a diameter of 4 micra.

A second instance of such spiral retraction of the fibre was found in the same region of the spinal cord of another specimen. In this the apparently functional fibre is found stretched taut from the sinus terminalis to a point as far forward as my sections go. Close against this fibre, however, there occurs a short stretch of freely lying fibre wound continuously in a fairly open spiral. I find it particularly difficult to account for the presence of this free piece of coiled fibre. It may of course be a remnant of the fibre broken some time previously, but in that case it is remarkable that it should have continued coiled.

The third case is that of the lamprey, above referred to, in which the fibre had broken free from the sub-commissural organ and had retracted into a knotted end in the central canal. For a short distance posterior to the free knotted end the fibre stretches backward as a simple spirally wound thread.

### The "Dachkern."

This remarkable group of large and conspicuous cells has been known under a variety of names, having been termed variously the "mesencephalic trigeminal nucleus" by Osborn ('88), the "nucleus magnocellularis" by Johnston ('01), the "nucleus magnocellularis tecti" by Edinger ('01), and the "mesencephalic nidulus of optic reflex cells" by Sargent ('04). The name "Dachkern" appears to have been first used by Rohon ('77), who applied it to the collection of large cells in the tectum mesencephali of Selachians, and though open, perhaps, to objection, is at least distinctive.

The nucleus has been described by many authors, and in nearly all classes of vertebrates. So far as I can find, however, it has never been identified in mammals, while in Teleosts it has been, as I believe, erroneously (see above) homologised with the torus longitudinalis by Sargent ('03 A).

In the Cyclostomes it was first recognised by Sargent ('04) in *Petromyzon marinus*, in which species he has described it (op. cit., p. 154) as consisting of two groups of large cells placed symmetrically on either side of, and at some little distance from, the median plane, each group containing from eight to twelve cells.

Sargent further claimed that he found these large cells in larval specimens of *P. planeri*, but, as I shall presently point out, it is evident that he has, in that ammocœte, altogether failed to interpret correctly the several structures in the roof of the brain, and from his figures it appears probable that the cells which he considers as the mesencephalic nidulus of optic reflex cells do not represent that nucleus at all.

That the cells of this nucleus are, however, present in all the members of the Petromyzontidæ is very probable, although possibly they become very reduced in some species. Thus Johnston was unable to find them in *Petromyzon* (*Lampetra*) *wilderi* ('02, p. 29) while I have not been able to identify them with certainty in ammocœtes of *Ichthyomyzon* (*Entosphenus*) *tridentatus* even in comparatively large specimens (up to 95 mm.). There is, however, in this species, a paired group of large, rather clear nuclei immediately external to the sub-commissural organ, which quite probably represent the "Dachkern."

The nucleus is present in *Geotria australis*, although the cells are not very well defined, in several of the series examined. The want of definition is partly due to the fading of the stain employed, but must be to a certain extent attributed to the fact that these cells appear to stain somewhat capriciously, the cytoplasm taking the stain well, in my experience, only when the tissue as a whole has been rather overstained.<sup>1</sup>

The large cells figured and referred to by Dendy ('07, fig.

<sup>1</sup> That this nucleus has been so long overlooked in the Cyclostomes may perhaps be attributed in some measure to this peculiarity. I believe, also, that they rarely, if ever, take up the silver impregnation of Golgi, which may possibly explain their alleged absence in *P. wilderi*.

3, and p. 17) do not form part of this "roof nucleus," but belong to the nucleus of the posterior commissure. It is in these laterally placed cells that Sargent finds the second source of axons for the fibre of Reissner. The cells of the "Dachkern" in *Geotria* lie comparatively near to the middle line, scattered among the fibres of the posterior commissure immediately dorsal to the sub-commissural organ.

It is principally in *Petromyzon fluviatilis*, however, that I have observed the "Dachkern" in this family. In this species it is moderately well developed, and is found to consist of a somewhat variable number of large cells which lie in the course of the posterior commissure. They are never very remote from the sub-commissural organ, against the inner border of which they mostly lie, either just above or to the outer side. The paired character of the nucleus is somewhat obscured, the cells lying sometimes closely adjacent in the middle line, sometimes trailing out in uneven lines with usually more cells upon one side than the other. Their number, all told, does not appear to exceed two dozen, but it was somewhat difficult to determine accurately their precise number, for, owing to their large size, each may appear in several consecutive sections. Some of the largest cells have a maximum long diameter of about 30 micra with a short diameter of approximately 12 micra. Their size and shape are very variable, but in all there is a large clear nucleus with well-marked chromatin network and one or two nucleoli. The diameter of the nucleus is usually about 10 or 11 micra.

That these cells, in the various *Petromyzontidæ* referred to, represent the "Dachkern" of higher vertebrates admits, I think, of no doubt, but that their axons constitute a part or the whole of Reissner's fibre I emphatically deny. It is, of course, possible that they are related to the cells of the sub-commissural organ, although I have not been able to establish this relation; their principal axons appear to pass with the fibres of the posterior commissure to that side of the brain remote from the cells from which they arise, but beyond that point I have not attempted to trace them.

The second source, according to Sargent, of the constituent fibrillæ of Reissner's fibre is the group of large cells lying somewhat lateral to the two halves of the sub-commissural organ. These, as already stated, are clearly part of the nucleus of the posterior commissure (Cf. Sargent, '04, pl. i, fig. 7, and Johnston, '07, fig. 132).

I have been quite unable to recognise the multipolar cells in the right habenular ganglion from which Sargent supposed a pre-commissural unpaired part of Reissner's fibre to be derived. As already repeatedly stated, I find the fibre arising as a structure, paired even in front of the posterior commissure, from the paired grooves of the sub-commissural organ, the two halves of the fibre remaining distinct in *Petromyzon fluviatilis* practically for the entire length of the mid-brain.

Although perhaps not directly related to the subject of this paper, I propose to make brief mention of certain other large and conspicuous nerve-cells in the roof of the brain. These are situated, in *Petromyzon fluviatilis*, high up on the walls of the hind brain some little way behind the cerebellum. They occur usually just beneath the ependymal epithelium, through which they often appear to bulge so as to lie exposed, practically, in the fourth ventricle. Traced backwards, these are seen to form a paired longitudinal series in which the two lines approach each other till that point is reached where the walls of the hind brain overarch and meet above the fourth ventricle. At this point the two converging lines of large cells become parallel and pass backward as that well-known paired tract of giant-cells which are such a conspicuous feature of the spinal cord of many of the lower vertebrates.

Thus in the common river lamprey these giant-cells do not end, as is said by Johnston ('02, p. 5) to be the case in the brook lamprey (*P. wilderi*), at the commissura infima, but continue forwards in the hind brain to about that point which coincides with the forward limit of the neural crests of the embryo.

In their size and staining reactions these cells in *P. fluviatilis* are practically identical with the large cells which form the "Dachkern" in the lampreys, and which also show evidence of a paired arrangement and have a similar situation on either side of the middle line. Further, the position and extent of the "Dachkern" in all vertebrates in which it is known to occur coincides closely with that of the forward isolated portion of the neural crests of the embryo. In the lampreys it is true that, owing to the roof of the mid-brain remaining largely membranous, the "Dachkern" is restricted to the anterior portion only of that region.

#### *Geotria australis.*

For the opportunity of studying the condition of Reissner's fibre in this species I am indebted to Professor Dendy, not only for the loan of his collection of sections through the brain of the velasia and ammocete stages of this lamprey, but also for placing at my disposal several well-preserved heads.

In all, I have examined eleven series of sections through the brain of the velasia, and one series (cut transversely) of the ammocete brain.

In his paper "On the Parietal Sense-Organs and Associated Structures in the New Zealand Lamprey (*Geotria australis*)," Dendy ('07) has briefly described the condition of Reissner's fibre in the brain, and its relation to the sub-commissural organ ("ependymal grooves"). In an earlier paper ('02) he had described the character of the sub-commissural organ in the ammocete.

These paired "ependymal grooves," which terminate, as such, at the hinder end of the posterior commissure, exactly as they do in *Petromyzon fluviatilis*, nevertheless differ from the grooves in that species in that their shallow lumina are presented almost ventrally (Dendy, '02, fig. 2) rather than mesially as in *P. fluviatilis* (figs. 34, 35, *s.c.o.*). Further, so closely, in *Geotria*, do these grooves approach each other in the middle line beneath the posterior com-

missure that the characteristic epithelium appears in the actual median section of series cut sagittally (Dendy, '07, pl. i, fig. 2). Immediately in front of the posterior commissure there is even an actual confluence for a short distance, the grooves presenting there the appearance of a single horse-shoe-shaped structure which lines the arching roof of the infra-pineal recess. Dendy ('07, pl. i, fig. 3) has figured the condition of the sub-commissural organ, as seen in transverse section, at a point immediately behind the infra-pineal recess. The condition of the sub-commissural organ in the infra-pineal recess foreshadows the condition which has become general along the entire length of the organ in many of the higher vertebrates (figs. 2, 3, 6, 8).

Ahlborn ('83), in his figures of *P. planeri*, has shown indistinctly (figs. 25, 26) what may be the paired ends of the grooves immediately behind the posterior commissure, and an unpaired median ependymal mass well developed beneath the posterior commissure. If this, indeed, represents the sub-commissural organ, we have here a further advance upon the incipient fusion of the grooves in *Geotria australis*. In *Petromyzon marinus*, on the other hand, the grooves appear, judging from Sargent's figures, to be even more widely separated than in *Petromyzon fluviatilis*.

Of the eleven series of sections through the brain of the velasia stage of *Geotria*, seven were cut sagittally and four transversely. In three of these the fibre could not be certainly made out, but the sections in two of these three cases were transverse, and in such sections Reissner's fibre is always particularly difficult to recognise.

In only one of the sagittally cut series of sections did I fail to find the fibre, and in this particular specimen the choroid plexus of the fourth ventricle had been dissected away before the sections were cut, and almost certainly the fibre was carried away at the same time.

In the eight brains in which Reissner's fibre was clearly to be seen it had retained its strictly normal position in only



one instance. It must, however, be remembered that the material had not been preserved for the study of this structure, the animals, in every case, having been decapitated. Under the circumstances, therefore, it is surprising that the fibre had been preserved in the normal position in any. The explanation, in this case, appears to be that, in some preliminary exposure of the brain, blood had found its way into the ventricles, and, clotting there, firmly secured the fibre from recoil. The condition of Reissner's fibre in this specimen is represented in fig. 57. The clot above referred to had filled the fourth ventricle and extended into the mesocœl, but the fibre can nevertheless be readily followed. It may be made out arising from the epithelium of the sub-commissural organ by many fine branches which run together into two principal factors. These unite, midway along the cavity of the midbrain, to form a single thread which passes backwards into the fourth ventricle, traversing a deep isthmie canal upon the ventral surface of the rhombo-mesencephalic fold. Behind the fourth ventricle the fibre may be followed to the end of that part of the *canalis centralis* included in the piece sectioned.

It is important to note that just as the two halves of the sub-commissural organ in *Geotria* are tending to become merged into a single median structure, so also the two halves of the fibre are more completely united. Their union occurs well forward in the mid-brain, whereas in *Petromyzon* and *Ichthyomyzon*,<sup>1</sup> in which the grooves are distinct and widely separated, the two factors of the fibre only lose their identity at a point far more posterior, beneath the rhombo-mesencephalic fold. The isthmie canal in these two latter forms may accordingly show more or less definite traces of a paired character, but in *Geotria* it is a simple median groove.

Three other series of sections also show particularly well the paired origin of the fibre in *Geotria*.

In one of these the whole length of the fibre, from the sub-commissural organ to the end of the piece of the spinal cord

<sup>1</sup> See below.



included, is actually contained in two adjoining sections. It has, however, sprung slightly, so that it lies (in the fourth ventricle) a little out of its proper position, as shown in fig. 20. This retraction has permitted some forward displacement of the point of union of the two halves of the fibre, these apparently meeting beneath the sub-commissural organ (fig. 58). In front of this point the paired fibres spread out into a brush-like end of lesser factors, which have broken free from their attachments and are slightly displaced in the sections. From the cells of the sub-commissural organ many constituent fibrillæ may be seen freely projecting, and these are particularly noticeable in the infra-pineal recess, as Dendy has described and figured ('07, p. 17, and fig. 6).

In another series the anterior end of the fibre closely resembles that just described, having also been slightly displaced anteriorly, so that in neither of these two can Reissner's fibre be actually traced to the cells of the sub-commissural organ. Posteriorly, however, the fibre in this series has recoiled into a tangled snarl which was apparently brought up at the hinder end of the isthmus canal.

In the last of these series the fibre has recoiled forward from the point of section to form a typical tangle beneath the posterior commissure as seen in the photomicrograph (fig. 19). In front of this point the paired factors of the fibre may be well seen.

In the four remaining series in which Reissner's fibre was made out it had in every case retracted into tightly wound knots, in the formation of which the fibre had become torn free from its attachment to the sub-commissural organ. In one case alone the snarl appeared as a mass of coiled and greatly swollen fibre immediately below the recessus infra-pinealis. In two others it occurred in similar form, but at the posterior border of the sub-commissural organ, while in the remaining instance it was less coiled but enormously swollen, and lying freely in the mesocœl. In this latter specimen the diameter of the piece measured varied between  $9.6\ \mu$  and  $18\ \mu$ . The normal diameter of the fibre in *Geotria*

appears to be between  $2\mu$  and  $3\mu$ , although in several instances it was found with a thickness of  $4.5\mu$ , even where there appeared to have been but slight retraction.

### Development.

Of the larval stages of *Petromyzon* I have had no material beyond the single series of transverse sections through the head of an ammocete 57 mm. long, this being the specimen in which the "ciliated grooves" of the sub-commissural organ were described by Dendy ('02).

In this series Reissner's fibre cannot be identified with certainty, nor could the large cells, said by Sargent to give rise to the fibre, be distinguished, although that author stated that he was able to discern these cells in ammocetes of *P. planeri* from 6 mm. to 10 mm. in length.

I have, however, been able to prepare and examine a fairly complete series of sections through the whole or parts of larvæ of *Ichthyomyzon* (*Entosphenus*) *tridentatus*, varying in length from 12 mm. to 105 mm., and while, so far as I could find, the ammocete of this genus does not differ markedly from that of *Petromyzon*, my findings are, nevertheless, markedly unlike those of Sargent, who examined *P. planeri*.

As, so far as I know, the nervous system of the ammocete of this lamprey has never hitherto been described, I propose to give a short account of the condition of the roof of the brain at certain stages in development, dealing simply with those parts of the brain with which this research is chiefly concerned.

### *Ichthyomyzon* (*Entosphenus*) *tridentatus*.

This lamprey inhabits the North Pacific Ocean, and is said to take the place in those waters of the very similar lamprey of the North Atlantic Ocean, *Petromyzon marinus*. Like *P. marinus*, it grows to a large size, and although the adult is not, I believe, easily obtainable, the ammocetes are found

plentifully during the early summer in certain of the rivers which drain the western slopes of the Rocky Mountains, and which seem to form the breeding-grounds of the species.

My specimens, which were collected for me by Mr. W. F. Allen from the Carmel R., California, were preserved entire in aceto-bichromate fluid.

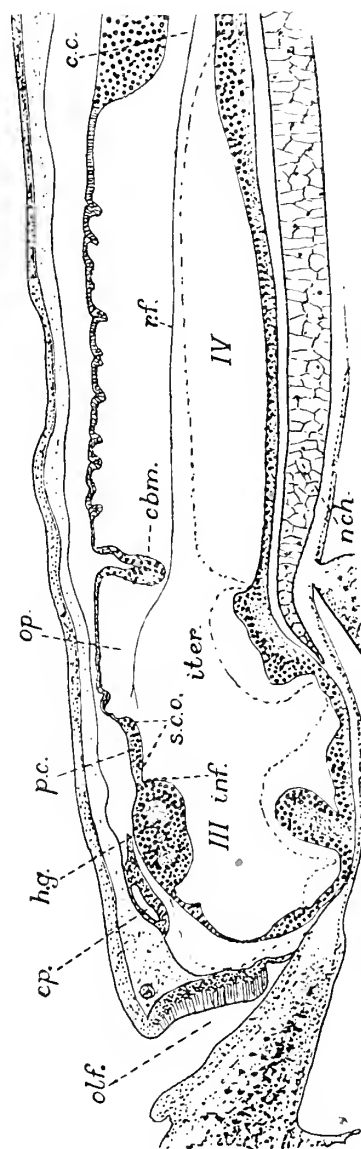
They are of all sizes from 12 mm. to 105 mm. The smallest specimens, I understand from Mr. Allen, were from two to three weeks' old, so that they have apparently a more rapid growth than the ammocetes of *P. planeri*, which were, according to Sargent, from 6 mm. to 10 mm. long at from twenty-six to thirty days after hatching. This difference is probably simply correlated with the considerable disparity in size of the adult animals of the two species and must not be lost sight of, for, as I do not know the ages of my various specimens, I shall be compelled to designate them by their respective lengths, which will probably be much in excess of those of ammocetes of river or lake lampreys of the same age and degree of development.

My smallest specimens included one of 12 mm. and another of 14 mm. which were cut transversely, and others 13 mm. (two) and 14.5 mm. which were cut sagittally.

In all of these the roof of the mid- and hind-brain is still almost entirely epithelial. The plica rhombo-mesencephalica is, however, already well marked, although still only one layer of cells in thickness. At its anterior end the membranous roof of the mid-brain (the future tela choroidea II) passes into the posterior commissure, which, even in my youngest specimens, is quite well defined. Although this has not, as yet, become downfolded to form the plica mesoprosencephalica, its hinder end has already come to lie at a slightly lower level than the developing choroid plexus (see Text-fig. 6).

In the fore-brain the greater part of the roof is occupied by the habenular ganglia, of which the right is enormously the larger. Above these lie the two epiphysial outgrowths, that of the right side being large, and having apparently,

TEXT-FIG. 6.



A median sagittal section through the brain, etc., of a 13 mm. Ammocoete of *Ichthyomyzon* (*Eutosphenus*) *tridentatus*. *cbm.*, Cerebellum. *c.c.*, Canalis centralis. *ep.*, Epiphysis. *hg.*, Habenular ganglion. *inf.*, Infra-pineal recess. *iter.*, Iter. *nch.*, Notochord. *olf.*, Olfactory opening. *pc.*, Posterior commissure. *pf.*, Reissner's fibre. *s.c.o.*, Sub-commissural organ. *III.*, Third ventricle. *IV.*, Fourth ventricle.

even at this early age, attained its full forward displacement relatively to other structures. All trace of the communication of its lumen with the third ventricle appears to be already obliterated, and as the posterior commissure lies immediately behind the habenular ganglia and at the same level, the recessus infrapinealis (Text-fig. 6, *inf.*) exists as a distinct space principally in that crevice which extends forwards from the posterior commissure for a short distance to the left of the habenular ganglion. Its anterior boundary is already defined by the left Meynert's bundle.

The ventral surface of the posterior commissure is covered by a conspicuous columnar epithelium (figs. 41, 45, *s. c. o.*), plainly disposed in a pair of longitudinal bands which meet in the mid-dorsal line and thus together form a single inverted trough upon the roof of the brain.

The cells of these epithelial bands attain their greatest length laterally, and their nuclei are there deep-seated. Towards the middle line the cells become shorter and have a length scarcely greater than that of their nuclei.

We have thus, at this early stage, a well-developed sub-commissural organ, the two halves of which are not, as yet, sharply marked off mesially from the ordinary ependymal epithelium. The half of the sub-commissural organ on the left side extends forwards into the infra-pineal recess slightly in advance of that upon the right side. Posteriorly the sub-commissural organ stretches, on both sides, the entire length of the posterior commissure, which, at this age, consists of a very thin band, indeed, of transversely coursing nerve-fibres (figs. 41, 45, *p. c.*).

Reissner's fibre may be made out, in sections cut sagittally, arising from the sub-commissural organ by several exceedingly delicate threads, which unite to form a single fibre in the aquæductus Sylvii. Thence it passes backwards as a fine thread freely through the fourth ventricle. It seems to lie against the ventral surface of the plica rhombomesencephalica, but nowhere penetrates the brain tissue, nor is there, at this stage, any trace of an isthmio groove for

its reception on the under surface of that fold (c.f. Text-fig. 6, *r.f.*).

It is exceedingly difficult to recognise the fibre in transverse sections of the brain; in the *canalis centralis* it may, however, be made out, but it is very fine; indeed, its diameter probably nowhere exceeds  $0.3\mu$ , which is its measurement at the hinder end of the fourth ventricle.

The next stage of which I have sections is represented by an embryo of 30 mm. cut sagittally, and a slightly older specimen (40 mm.) cut transversely.

In these, beyond the increase in size of the various parts, there has not been a great deal of change (figs. 42, 46). The choroid plexus of the mid-brain has grown forward so that it now overlies the hinder part of the posterior commissure, and has a well-marked ventrally projecting median fold, much as Dendy has described ('02) for the ammocœte of *Geotria*. (This fold was barely indicated in my smaller specimens.) The posterior commissure still shows no indication of any downfolding, and remains extended horizontally in an antero-posterior direction, on a level with the top of the habenular ganglia.

The sub-commissural organ has increased considerably in size and its two halves are now seen to be somewhat widely separated (fig. 42, *s. c. o.*). At its anterior end the left half of the organ extends forward a short distance in advance of that upon the right side. Posteriorly the specialised ependymal epithelium now extends a little way behind the posterior commissure upon the side walls of the mid-brain in a manner exactly recalling the condition of the ammocœte described and figured by Dendy ('02, p. 490).

Reissner's fibre, too, shows a marked increase in size, having, in the 30 mm. specimen, a diameter of approximately  $0.8\mu$ , measured in the fourth ventricle. The lesser fibres have joined up beneath the posterior commissure into a pair of fibres which remain distinct for some distance, and seem to unite to constitute the single fibre just anterior to the rhombo-mesencephalic fold. In transverse sections the

fibre, though not easily followed continuously through the brain-ventricles, may nevertheless be followed under the cerebellum, and becomes easy to trace in the *canalis centralis* of the spinal cord, where it has a diameter of almost  $1\mu$ .

In an *aminocæte* 65 mm. in length, the only important feature to note is the development of the isthmic canal upon the ventral surface of the rhombo-mesencephalic fold. This canal in this specimen shows distinct traces of a paired character (fig. 44, *i. c.*).

The right and left halves of the sub-commissural organ have become still more widely separated, and each is now slightly hollowed out to form a groove, the ventro-lateral borders of which are marked off very distinctly from the general ventricular epithelium.

Owing probably to the larger size of this specimen, penetration by the fixing fluid seems to have been less rapid, and the fibre is not quite so well preserved. It has, in the *canalis centralis*, a diameter of barely  $1\mu$ .

Another specimen, 95 mm. long, cut, like the last, transversely, shows some advance upon the condition just described. The sub-commissural organ consists now of a pair of very definite grooves, each somewhat crescentic in transverse section (fig. 43, *s. c. o.*). As in all the other specimens examined, the sub-commissural organ begins in front on the left side, in the cleft between the habenular ganglion and the optic thalamus, and on the right side, somewhat further back, behind the habenular ganglion. The two grooves lie widely apart, have a nearly mesial presentation, and both extend very slightly behind the posterior commissure.

This latter structure has enlarged considerably, and owing to the marked growth of the habenular ganglia, its upper surface now lies at a somewhat lower level than the dorsal surface of those ganglia. Further, from behind, the *tela choroidea II* has continued to grow forward and upward. The posterior commissure, therefore, being left behind by the more rapid growth of the adjacent structures,



seems to be becoming downfolded as the plica meso-prosencephalica. It is, however, still a comparatively thin band of fibres, the upper and lower surfaces of which, as seen in sagittal section, are horizontal.

Upon the ventricular surface of the sub-commissural organ in some sections fairly numerous fibrillæ of Reissner's fibre may be distinguished. The fibre itself passes backwards beneath the rhombo-mesencephalic fold as a single thread, which traverses a deep isthmie canal upon the ventral surface of that fold. In this canal Reissner's fibre lies quite freely. The diameter of the fibre in the fourth ventricle of this specimen is little more than  $1\mu$ .

The study of the posterior end of the fibre is attended with much more difficulty on account of the exceedingly minute lumen of the *canalis centralis* towards its hinder end.

The spinal cord has been stated to arise in Cyclostomes and in Teleosts as a solid cord, its lumen appearing subsequently. In my youngest specimens the lumen has already appeared, and the spinal cord has assumed the appearance characteristic of early vertebrate embryos. In its hinder part the spinal cord is almost cylindrical, and has accordingly, in transverse sections, a nearly circular outline. Owing to the thickness of the walls laterally and to the relative thinness of the median zone the *canalis centralis* appears in such sections as a narrow vertical cleft, which is almost obliterated near the middle of its height. Below it widens into a tiny circular space, and sometimes similarly widens slightly above (fig. 47, *c. c.*).

In the 12 mm. larva this central canal has a maximum transverse diameter of little more than  $4\mu$ , measured at a point about half a millimetre from the end of the body. Forward of that point the lumen is occasionally blocked by an intrusive cell, but behind the canal is entirely free from any such contents.

The *canalis centralis* extends to the hind end of the body, appearing even in the section last but two of this series, which was cut transversely in sections  $10\mu$  in thickness.

In the last few sections the spinal cord narrows into a *filum terminale* (fig. 48), in which the *canalis centralis* gradually widens out into a space, almost square in transverse section, which is enclosed only by a columnar ependymal epithelium, and which must be the *sinus terminalis* (fig. 49, *s. t.*).

This terminal chamber extends through six sections only (60 micra), and has a maximum diameter of  $8\mu$ . It is absolutely free from contained cells of any kind in my youngest specimens.

In many sections through the *canalis centralis* Reissner's fibre can be made out as a very minute dark dot, and may be traced backwards to the *sinus terminalis*, where it is lost.

From what has been stated above of the size and character of the lumen of the central canal at this age, it will be obvious that it is scarcely practicable to trace the fibre in sagittal sections through the tail region, for even where it happened that the plane of the sections was exactly sagittal, the canal was invariably contained within the thickness of a single section. Only in the *sinus terminalis*, therefore, could the lumen be satisfactorily examined, and in this, in both of the 13 mm. specimens and also in the 14.5 mm. specimen, Reissner's fibre may be made out as an extremely delicate thread, which in the longer of these three specimens ends in a swollen knob. This knob appears to be a mass of coagulum (fig. 50, *t. p.*), and in it may be distinguished the remains of nuclei. From the condition, however, in one of the 13 mm. specimens, it would appear that this knob may really represent the terminal plug, which would then apparently have a cellular origin.

In both cases this terminal plug, if it be such, has become detached, and in the smaller specimen it has also apparently been displaced slightly in the preparation of the sections. In both also the *sinus terminalis* opens posteriorly by a small gap in the ependymal epithelium, at a point external to which lies a group of indifferent cells (Fig. 50, *m. t.*) which represent,

probably, the "massa terminale" of Sterzi ('07, p. 303). It seems probable that the terminal plug was, in life, inserted in this gap. Owing to its shape (almost hour-glass in form in transverse sections) the central canal is, at a point just anterior to the *sinus terminalis*, cut twice in sagittal sections, the upper and lower portions of the canal being separated by the bulging masses of the side walls. Reissner's fibre lies always in the lower canal (fig. 48, *c. c.*).<sup>1</sup>

In older ammocetes, however, the central canal has become considerably larger, and the fibre of Reissner may be much more readily traced. As it traverses the central canal it is seen to be joined at short intervals by cilia from the ependymal cells (fig. 56). These attachments seem to be quite strong, for the fibre has deviated slightly from a straight line, and instead of running centrally along the canal has a gentle zig-zag course as though held firmly here and there. The particular instance figured was that observed in the central canal in the tail region of a 34 mm. ammocete, but such a condition of the fibre may be seen in well-preserved material of practically all larval forms, in which, of course, the fibre is still relatively slight. In still older specimens, where the fibre attains a greater size, it appears to follow a more nearly straight course at or near the centre of the canal.

In a larva of 36 mm. the *sinus terminalis* lies immediately dorsal to the extremity of the notochord. It is here an ovoid space almost surrounded by the ependymal epithelium, which is, however, incomplete dorso-posteriorly. Into the gap thus left there is fitted a conical mass, the terminal plug (fig. 53, *t. p.*), from the apex of which, projecting antero-ventrally, Reissner's fibre may be made out running forward.

Another specimen, somewhat older (42 mm. in length), also shows Reissner's fibre ending in a terminal plug (Fig. 51, *t. p.*), which seems to be continuous with the mesenchymatous tissue which lies immediately behind the terminal neural pore.

<sup>1</sup> Cf. the condition of the central canal of *Myxine* and *Bdellostoma* described below.

The terminal plug still retains a postero-dorsal insertion. At this stage the *canalis centralis* is seen to have increased considerably in size, but immediately anterior to the extremity of the *filum terminale* it appears to become more shallow, then deepens and widens into the *sinus terminalis*. Thus, in this particular specimen, the terminal sinus appears conical rather than ovoid in shape. It is probable, however, that the posterior portion of its wall, constituted by the meningeal sheath, has collapsed somewhat, for in a slightly larger specimen (52 mm.) the arrangement of the various structures is precisely similar, excepting that the meningeal portion bulges upwards and outwards, dome-like. It will be noticed that this meningeal portion of the wall of the sinus now lies posteriorly instead of postero-dorsally.

In older larvæ the neural tube has apparently outgrown the supporting notochord and has become turned down behind it. In fig. 52 the posterior end of the spinal cord of a 75 mm. ammocœte is shown partly bent, the bend occurring at the anterior end of the *sinus terminalis*, while in the oldest larva that I have examined (105 mm.), the terminal neural pore has become ventrally directed, and lies below the level of the notochord (figs. 14, 54).

Unfortunately, in none of these older ammocœtes examined (all of which were preserved entire) has the fibre remained in its normal position, but it is broken and ends in a tangle in the *sinus terminalis*.

Such tangles have been found in the terminal sinns of specimens of 65 mm. (fig. 55), 75 mm. (fig. 52), 90 mm. (fig. 15), 95 mm. and 105 mm. (figs. 14, 54). The amount of retracted fibre varies considerably in the different specimens, forming in the cases of the 90 mm. and 95 mm. ammocœtes conspicuous masses of spirally twisted fibre that almost fill the terminal sinus. The condition of the fibre in the 65 mm. specimen is noteworthy, for in this case the recoil seems to have pulled into the terminal sinus some of the fibrous meningeal tissue. The terminal plug cannot be distinguished, being overlain, presumably, by the tangle of Reissner's fibre.

Figure 55 represents the fibre of Reissner emerging from this tangle, and shows also some of the fibrous tissue of the sheath drawn into the terminal sinus. In all of these cases the fibre is enormously swollen.

On the other hand, in the specimens of 75 mm. and 105 mm. the recoils were evidently much less extensive, the break in the fibre doubtless having occurred very far posteriorly. In the latter specimen a quantity of lymphoid matter has intruded through the terminal neural pore, and with this Reissner's fibre (which is not greatly coiled) seems to have become entangled. It may possibly be that we have here a case of incipient regeneration.

In the brain of the ammocœte of *Geotria* the sub-commissural organ is well developed. Its condition has already been described and figured by Dendy ('02, figs. 1, 2), and it differs from that of the ammocœte of *Ichthyomyzon* (*Entosphenus*) *tridentatus* of corresponding age in little but that its two halves are more nearly apposed in the middle line, and that in consequence of this median position the lumina of the grooves are presented almost directly ventrally.

The cilia which clothe the ventricular surface of this organ are short and close set, and amongst them are some longer undoubted fibrillæ of Reissner's fibre. I cannot, however, certainly identify the fibre itself in this region, but beneath the rhombo-mesencephalic fold there is a shallow isthmie canal in which Reissner's fibre may be made out. In the *canalis centralis* of the spinal cord it can be readily traced. Of the tail of this species I have had no material, so that nothing can be stated as to the condition of the fibre and its relation to the *sinus terminalis*.

#### Critical Discussion.

##### (a) The Sub-commissural Organ in the Adult.

It has already been pointed out above that in *Petromyzon marinus*, *Petromyzon fluviatilis*, *Ichthyomyzon* (*Entosphenus*) *tridentatus*, *Geotria australis* and

*Petromyzon planeri* (?) we have apparently a progressively arranged series in the evolution of the sub-commissural organ.

I have further shown that in all the members of this family that I have examined this organ ends abruptly at the hinder border of the posterior commissure, or quickly fades away behind it into the general ependymal epithelium of the iter.

For *Petromyzon marinus*, however, Sargent has given a widely different account ('04), stating that the paired grooves in that species curve around the hinder border of the posterior commissure and pass dorsally (cf. his text-figure A, p. 151) to the anterior extremity of the mesocœl, but, as already remarked, his other figures (Pl. 1, figs. 6 and 7) do not at all bear out his statements. I have not myself examined sections through the brain of this particular lamprey, but assuming that the figures given by Sterzi ('07) represent correctly the extent of the choroid plexus of the mid-brain and its relations to the posterior commissure, it is obvious that the entire dorsal surface of the latter structure lies ventral to this choroid plexus. That being so, the space overlying the posterior commissure, as represented by Sargent in his fig. 7, can only be part of the forward extension of the mesocœl. The two halves of the sub-commissural organ (ependymal grooves) should, therefore, appear in the figure upon the dorsal surface of the posterior commissure if, as Sargent states ('04, p. 152), "Posteriorly both grooves extend . . . downward under the posterior commissure, at the same time coming nearer together. Here they curve around the commissure . . . and continue cephalad into the recessus of the mesocœl above the commissure and thence into its anterior horns. The horns of the recessus are completely lined by this characteristic ependyma. A transverse section through the anterior part of the posterior commissure shows the horns of the recessus as small circular orifices with thick walls composed of this radiating ependyma (pl. i, fig. 6, *rec. 1*')."

The last part of this statement is especially difficult to

reconcile with his fig. 6 (pl. i), for he there shows this recess lying beneath the posterior commissure instead of above it, as described.

Not only are Sargent's statements and figures thus conflicting, but I find nothing in any of the lampreys which I have examined which will throw any light upon the condition which Sargent figures (pl. i, fig. 6)—nothing, that is, of the nature of a postero-dorsal continuation of the sub-commissural organ behind and above the posterior commissure.

I have, however, described paired "diacœlic recesses" (see above) which are shallow pockets bulging caudally from the recessus infrapinealis to overlie slightly the posterior commissure from in front (fig. 35). These recesses are completely lined with the characteristic epithelium of the sub-commissural organ, and in transverse sections through the anterior border of the posterior commissure do present an appearance not unlike that figured by Sargent for the mesocœlic recess. These diacœlic recesses of *Petromyzon fluviatilis* are almost certainly identical with the "recessi postabemplari" of Sterzi, which are apparently particularly well developed in *P. marinus*. An explanation, therefore, which suggests itself in connection with this conflict of statement and figure in Sargent's paper, is that that author may have mistaken the diacœlic recess opening forwardly into the infrapineal recess for a mesocœlic recess opening backwardly beneath the choroid plexus of the mid-brain. An accidental misplacement of a few sections might easily give rise to such a confusion.

#### (b) Development of the Sub-commissural Organ and Reissner's Fibre.

Sargent's account, too, of the development of the fibre is incorrect, which I can only suppose to be due to the fact that he did not recognise the embryonic sub-commissural organ, having failed once again to correctly identify the posterior commissure.

A comparison of Sargent's figures of the brain of the



ammocœte of *Petromyzon planeri* with those of Sterzi ('07, figs. 99 and 124-6) for the same ammocœte, and also of that of *P. fluviatilis*; or with my figures (figs. 45, 46, and Text-fig. 6) of the corresponding structures in *Ichthyomyzon* (*Entosphenus*) *tridentatus* will, I think, convince the reader that what Sargent has named the posterior commissure ('04, pl. i, fig. 2) is undoubtedly the superior (habenular) commissure, while that part of the brain which he labels *tectum opticum* clearly represents the region of the posterior commissure.

Indeed, a comparison of Sargent's own figures (*op. cit.*, pl. i, figs. 2 and 4) bears out this conclusion. In his fig. 4 the epiphysis is seen, cut in transverse section, dorsal to the habenular ganglion, which is perfectly correct for very young larvæ (*cf.* Sterzi, *op. cit.*, fig. 122). In these young specimens it may also extend backwards to a slight extent, dorsal to the posterior commissure. It continues, however, to grow forward, and, as I have pointed out above, has already, even in my youngest specimens, attained its full forward displacement relative to the other parts of the brain, lying above the anterior region of the dorsal sac.

In his fig. 2, however, Sargent represents it extending dorsally far backwards, behind the posterior commissure, and altogether posterior to the habenular ganglion, a quite unnatural position if the parts were correctly identified. Further, the posterior commissure reaches a size<sup>1</sup> and shape comparable to that of the body so labelled in Sargent's figures only comparatively late in development, by which time the epiphysis has acquired its well-developed eye-like appearance and has an elongated stalk. The right habenular ganglion, on the other hand, has a very precocious development and early reaches a large size, presenting at this age precisely the appearance and relations of the structure which Sargent has named the posterior commissure.

<sup>1</sup> Even in larvæ of *Ichthyomyzon* (*Entosphenus*) *tridentatus* 95 mm. long, the posterior commissure had not such a relatively considerable thickness as compared with its length.

A study of the figures of the development of the brain of any lamprey will confirm these statements.

The existence, too, of a well-developed median brain nucleus in the roof of the brain well behind the posterior commissure (Sargent, '04, pl. i, fig. 3) is altogether incredible, for, as already pointed out, the whole of this region of the brain-wall, at a very early stage indeed, has taken on its permanent character of a cubical epithelium lining the intra-cerebral surface of the tela choroidea. Again, Sargent's description of the position of the "optic reflex cells" behind the posterior commissure in the larva is wholly incompatible with the fact of the position of this nucleus in the adult at a point well forward, dorsal and lateral to the posterior commissure.

All these difficulties disappear when the correction which I have indicated above is made in the labelling of the several parts shown in Sargent's figures, and that author's descriptions, thus amended, would agree very closely with my own observations recorded above.

Sargent is thus, I believe, entirely mistaken in homologising the layer of large cells bulging downwards into the iter with the cells of the "Dachkern" (his "nidulus of optic reflex cells"). The cells of the "Dachkern" in the adult are not so superficial in position and are comparatively few in number (less than two dozen all told), whereas the cells in the group beneath the true posterior commissure in the larva are very numerous (cf. Sargent's figs. 1-3), and even so some of them still appear to be undergoing division. Although, on a casual inspection, the nuclei of these cells seem in the sections to form part of a many-layered structure, a closer examination reveals the fact that this appearance is misleading and is the result merely of the accommodation of the nuclei of a closely crowded group of attenuated cells which are actually arranged to form a single layer investing the under surface of the posterior commissure.

From the ventricular surface of certain of these cells a large cilium (or more probably a group of coalesced cilia)

already projects freely into the ventricle, these being the constituent fibrillæ of Reissner's fibre (Fig. 45, *fb*). For the greater part the cells are still undifferentiated; they are destined, however, to give rise to the cells of the sub-commissural organ, while from their cell bodies grow out the constituent fibrillæ of Reissner's fibre. I am not prepared, of course, to say that no single cell in this group depicted by Sargent in his fig. 3 could form part of the future "Daehkern," but that such cells, if present, send axons into the ventricle, I entirely disbelieve.

(c) Reissner's Fibre in the Brain-ventricles.

In two or three other particulars my observations upon Reissner's fibre in the lamprey are completely at variance with those recorded by Sargent. Thus, in no single instance in any specimen of the *Petromyzontidæ* did I find any part of Reissner's fibre arising from the dorsal surface of the posterior commissure, and I am utterly at a loss to explain Sargent's statements that it arises there. If the sub-commissural organ extends onto that surface there is, of course, no reason why the fibre should not receive factors from that region, but, as I have pointed out above, Sargent's figures do not convey the impression that such a dorso-posterior extension of the sub-commissural organ has any real existence. Sargent's statements, however, imply that not merely does the fibre arise in part from that surface, but that these portions of the fibre constitute its main trunks.

Indeed, the factor from the left side of the brain, according to Sargent, springs wholly from the dorsal aspect of the posterior commissure ('04, pp. 155-6). The part of the fibre which he finds in the diacœle arises, he claims, wholly from the habenular ganglion of the right side, and is collected into a single factor which joins the main right trunk behind the posterior commissure. He could find, he states, no fibre upon the left side traceable to the region of the left habenular ganglion.

Thus, ignoring even the vital differences existing between Sargent's account of the origin of the factors of Reissner's fibre from nerve-cells and my own description of its origin from ependymal cells, I am still unable to reconcile Sargent's description of the course of Reissner's fibre in *Petromyzon marinus* with my own observations upon its path in other lampreys, and, indeed, in vertebrates of other groups.

In the three species of lamprey of which I have examined the brain, Reissner's fibre invariably takes its origin in the infrapineal recess by the union of delicate fibrillæ into a pair of fine threads, which receive constant accessions in their nearly parallel course beneath the posterior commissure. Of constituent fibrillæ from the dorsal surface of the commissure there are none. The paired fibres converge in the mesocœl, and coalesce into a median structure either beneath the rhombo-mesencephalic fold (*P. fluviatilis*) or at some point in front of that (*Ichthyomyzon* and *Geotria*).

Again, in not one of more than thirty lamprey brains (larval and adult) which I have studied have I found the fibre embedded in the brain-tissue of the rhombo-mesencephalic fold as described by Sargent ('04, p. 156). He states: "The fibre passes through this portion of the brain and the basal part of the cerebellum in the median plane a little dorsal to the passage connecting the third and fourth ventricles," and "in both the transverse and sagittal sections that I have studied Reissner's fibre has been found always to enter the right tuberculum acusticum (fig. A)."

In the former statement Sargent has confirmed the description presented by Studnička ('99) of this part of the course of the fibre in *P. planeri*, the latter author having stated that he found Reissner's fibre surrounded by the brain-substance in his two series of sagittally cut sections of the adult brain.

That in the lampreys (as, indeed, in many vertebrates) Reissner's fibre appears in sagittal sections to penetrate the ependymal tissue of the rhombo-mesencephalic fold, I am perfectly willing to admit. That it actually does not do so I am firmly persuaded. Sections cut absolutely truly in the

sagittal plane are not easily prepared, and a very slight obliquity might cause the open isthmic canal to appear as an exceedingly fine tube scarcely larger than the fibre traversing it. Even if the sections were, however, cut perfectly truly, the whole isthmic canal might easily lie within the thickness of a single section, for the full width of the double canal at the point shown in my fig. 10 is less than  $6\mu$ , while behind that point it narrows considerably.

Thus, to determine with certainty whether or no the fibre lies freely, it is necessary to examine sections cut transversely in this region. Even so, it does not follow that the sections so cut will show the fibre, for unless they are prepared with especial care it is more than likely that pieces of fibre may become displaced or lost. It is, therefore, of interest that in the one series which Studnička examined which was cut transversely he found the fibre free ('99 p. 7). This particular series of sections happened to be one through the brain of an ammocœte, and Studnička concluded that the inclusion of the fibre within the substance of the plica rhombomesencephalica must take place later in life as a consequence of the downgrowth of the brain in this region.

While, of course, there is nothing inherently improbable in the supposition that the ependymal epithelium of the edges of the isthmic canal should fuse ventrally, beneath the fibre, to form a tube open at either end,<sup>1</sup> I see no reason to suppose that such a condition actually arises in the Petromyzontidæ, and I regard Sargent's statements merely as a result of his misinterpretation of the condition observed in sagittal sections.

I have described the occurrence of large snarls in the recessus infrapinealis and beneath the posterior commissure (Geotria), showing that the fibre, when cut posteriorly, is free to spring forward from its broken end in the central canal of the spinal cord, or equally, breaking free from its attachment to the sub-commissural organ, to spring

<sup>1</sup> Such an inclusion of the fibre within a more or less wide isthmic canal tube actually occurs in the Myxinoids (see below).

backward into the fourth ventricle (*Petromyzon*). Nothing of this sort could happen if the fibre were buried in the brain-substance of the rhombo-mesencephalic fold.

(d) The Sinus Terminalis.

As to the universal occurrence of a sinus terminalis there appears to be some diversity of opinion. Both Retzius ('95) and Sterzi ('07) seem to have found this terminal dilatation in some specimens of *Petromyzon marinus* and *P. fluvialis*; Studnicka ('95) found it invariably present in *P. fluvialis* and *P. planeri*, but he denied that it was present in ammocetes less than 10 cm. long. Schäffer ('01, fide Sterzi) apparently found this terminal dilatation to be constant in all ammocetes. Sargent refers to the sinus terminalis of the ammocete (6-10 mm. in length), but gives no figure, and his figure of the adult terminal sinus is incomplete. I have been able to recognise the sinus terminalis as a dilatation of the terminal portion of the central canal in all of the ammocetes of *Ichthyomyzon* which I have examined, but it is only in specimens of about 40 mm. and upwards that there is an actual bulbous enlargement of the end of the filum terminale. The change of position which this terminal sinus undergoes during larval life is noteworthy. In the young specimens the neural tube stretches wholly dorsal to the notochord and the sinus terminalis lies immediately dorsal to its extremity. The actual terminal neural pore is dorsally directed, and the hinder wall of the sinus terminalis is continuous with the terminal mass of neurenteric cells which caps the end of the notochord. As growth proceeds the sinus terminalis enlarges and begins to turn downward behind the notochord, ultimately coming to occupy the place of the terminal mass. This is not due, however, to an extension of the lumen of the neural tube into the midst of this mass of neurenteric cells as a continuation of the process by which the central canal is said to have arisen. It is to be



attributed rather to the unequal growth of dorsal and ventral surfaces of the filum terminale. The terminal neurenteric mass of cells disappears, and growth appears to go on much more rapidly upon the dorsal surface of the filum terminale. In this way the sinus terminalis becomes carried downward behind the notochord, and as a consequence of this unequal growth the terminal neural pore undergoes a complete change of position. Primarily directed dorsally,<sup>1</sup> it has in turn a postero-dorsal, a posterior and a postero-ventral presentation, and finally takes up an actual ventral presentation.

The sinus terminalis is present in those adult specimens of which I have been able to examine undamaged material, and in every case the terminal neural pore has the ventral position.

#### (e) Reissner's Fibre in the Sinus Terminalis.

In the manner of the ending of the fibre posteriorly, too, the condition in *Petromyzon fluviatilis* and larval *Ichthyomyzon tridentatus* is markedly unlike that which Sargent has described for larval *P. marinus* ('01), adult *P. marinus* and larval *P. planeri* ('04).

According to that author ('04, p. 149) the posterior part of Reissner's fibre arises, in early larval development, by the coalescence of forwardly growing axons from a group of "posterior canal cells" situated wholly within the terminal sinus. This statement was made in the first instance of larval *P. marinus* ('01, p. 448), probably in mistake, for subsequently Sargent states that his investigations of the development of the optic reflex apparatus in Cyclostomes were "confined to the larval stages of *Petromyzon planeri*" ('04, p. 149).

<sup>1</sup> It is of interest to note that in *Amphioxus* (in which I have been unable to determine with certainty the occurrence of Reissner's fibre, although I believe it to be present) there is a sinus terminalis dorsal to the notochord, and with an apparent terminal neural pore, directed, as in young ammocetes, dorsally.



Of adult material, Sargent states that the hinder part of the spinal cord of a single specimen of *P. marinus* was alone examined. From the account which he gives of the preparation of the material, I suspect that the actual sinus terminalis was, partially at least, destroyed, for, as I have already pointed out, the hinder wall of the terminal sinus lies so closely (in *P. fluviatilis*) beneath the skin that the removal of that and the adjoining muscles without injury to the sinus terminalis is a matter of extreme difficulty. In this suspicion I am confirmed by the fact that Sargent offers no description of the terminal chamber, while his figure ('04, pl. i, fig. 3), which professes to represent the terminal sinus, clearly does not do so.

A careful study of Sargent's work shows that, apart from this single tail of *Petromyzon marinus*, he nowhere records the examination of the condition of the sinus terminalis in any other adult vertebrate.

Nevertheless, on the strength of an examination of the condition of this single, almost certainly damaged specimen, he twice ventures ('00, '04) to controvert the account given by Studnička of the mode of ending of Reissner's fibre in *Petromyzon* and *Myxine*. He says ('04, p. 160), "Studnička's statement that the end of Reissner's fibre passes out of the sinus of the ventriculus terminalis and into the surrounding lymph-space is so at variance with all my observations that I must believe the appearance he so interprets was accidental and due to the disturbed and abnormal condition of the fibre in his preparations." (The spaced type is mine.)

As a matter of fact, the account given by Studnička, which was based on the study of a large number of series of sections of several different species, is substantially correct so far as it goes. It is true that the coiled condition of the fibre is probably not normal but due to the recoil of the fibre following some breakage. It is nevertheless a condition very frequently to be observed. Whether, in the case of Studnička's material, this breakage was due to the cutting of the fibre in the fresh

condition in the preparation of the material, or whether the material was hardened entire and the recoil was the result of some breakage of the fibre in life, Studnička in his paper affords no clue. The condition recorded by Sargent was certainly produced artificially by his treatment of his material, and his description of the end of the fibre in adult *Petro-myzon* thus becomes nothing more than an account of a tangle in the hinder part of the central canal, which would apply equally well to a tangle occurring at any point (cf. my figure, '12, fig. 3).

Whether his failure to observe its proper point of ending in the sinus terminalis was due to his failure to trace the *filum terminale* to its end, or, as I think more probable, because the actual extremity of the central canal was lost in the preparation of his material, it is not now possible to determine.

Notwithstanding that his description thus betrays the fact that he himself had failed to discover the normal ending of the fibre in the adult sinus terminalis, he nevertheless disputes the correctness of Studnička's statement that the fibre emerges from the end of the central canal into the lymph chamber which encloses the end of the neural tube.

For this altogether unwarrantable proceeding I can find no excuse. Even if Sargent had correctly observed the condition of the end of the fibre in the ammocœte, he was not justified in an assumption that such a condition must necessarily persist in the adult. As a matter of fact, I believe he is also mistaken in his account of the larval condition. I have not examined, it is true, larval material of *P. planeri*, but in a very complete series of ammocœtes of *Ichthyomyzon tridentatus*, ranging from 12 mm. to 105 mm. in length, I find nothing to confirm the account given by Sargent of the condition of the posterior end of the fibre in larval Cyclostomes.

The sinus terminalis, as already stated, is present in this species as a terminal dilatation of the central canal, even in my youngest ammocœtes. Behind it is blocked by a solid

cellular mass (the terminal mass of Sterzi), which is stated by Goette ('90, fide Favaro) to represent a solid neurenteric connection. Even at this age Reissner's fibre is visible in this region, and ends, apparently, in a conical terminal plug, the origin of which I could not determine. Although my smallest specimens were actually longer than those studied by Sargent, I believe, judging from the condition of the *canalis centralis*, that they were really younger than his specimens.

As I have pointed out above, it is exceedingly difficult to follow the fibre forwards from this point in the smallest specimens on account of the minute size of the central canal. In slightly larger specimens it is, however, possible to trace the fibre as a continuous thread from its origin in the brain to its end in the terminal sinus, but from first to last I could find no indication of the existence of posterior canal cells.<sup>1</sup> In one or two instances intrusive ependymal cells were found in the central canal, but none of these ever happened to occur in the *sinus terminalis*.

#### IV. REISSNER'S FIBRE AND THE SUB-COMMISSURAL ORGAN IN THE MYXINOIDEI.

The heads only of some half dozen *Bdellostoma* (*Polistotrema*) *stouti*, the common hagfish of the Californian coasts, were sent to me by Mr. Wm. F. Allen. Of these I have examined the brains of four in serial section. I have also examined a series of sections through the head of a single specimen of the Cape species of hagfish, *Bdellostoma cirrhatum* (*B. forsteri*). The sections of this last, however, were transverse and quite thick. Further, the stain employed was not the best for the purpose of this investigation. The descriptions, therefore, of the condition in

<sup>1</sup> An examination of a large number of specimens of other larval forms (an account of which will be given in subsequent parts) has given me similar negative results.

*Bdellostoma* will relate to *B. stouti* unless otherwise expressly stated in the text.

Of *Myxine glutinosa* I have had a dozen entire specimens, but of these only two were preserved with any care, being put entire into strong formalin immediately after their capture. The remainder were simply thrown into spirit and had almost certainly been dead for some time before preservation.

It has been remarked by several writers who have dealt with the morphology of the central nervous system of Myxinoids that there is an extraordinary range of variation to be met with in the brains of different individuals. This, my own observations, made upon eight Myxinoid brains, quite confirm.

In particular, this variation is most marked in the extent to which the brain-ventricles have become reduced. In some specimens the cavities of the brain are relatively spacious, while in others they are enormously reduced, and in parts even altogether obsolete.<sup>1</sup>

Those portions of the lumen of the brain which are traversed normally by Reissner's fibre are, however, least variable and are never wholly obliterated, a fact, I believe, not without considerable significance as to the importance of that structure.

My conclusions as to the homologies of the cavities of the mid- and hind-brain agree very nearly with those already put forward by Sterzi ('07). They were, however, arrived at quite independently, and were based principally upon a consideration of the relations of Reissner's fibre and the sub-commissural organ, to which Sterzi, whose work came into my hands only recently, apparently makes no reference. Indeed, it is a surprising fact that of the several workers who have concerned themselves with the histology of the Myxinoid

<sup>1</sup> This may result, I believe, from a continuance late into life of the process of thickening of the walls of the brain, their apposition and fusion, the differences observed then being merely a matter of age.

brain, practically all have failed to refer to the extraordinary development of the ependymal epithelium (of the sub-commissural organ), and to its remarkable distribution upon the ventricular walls.

*Bdellostoma (Polistotrema) stonti.*

In the brain of this animal the reduction of the ventricular spaces has not, in my specimens at least, proceeded so far as is the rule in *Myxine*. Nevertheless, practically the whole of the third ventricle may be obliterated—the infundibular cavity alone persisting—for the so-called “trigonum cinereum,” though frequently present, is apparently not always to be made out. Where the reduction has progressed least there may be found a canal, the “*canalis connectens*” of Holm ('01), which runs upward from the infundibular cavity to join the mesocœlic cavity. Into the “*canalis connectens*” there may open a canal which runs backwards from the trigonum cinereum. In others, this *canalis connectens* may be represented merely by a broken chain of isolated spaces, or even marked only by a band, of varying width, of scattered nuclei, the remains of the cells of the ependymal epithelium which, earlier in life, lined such spaces. Even these traces of a one-time connection may be altogether lost.

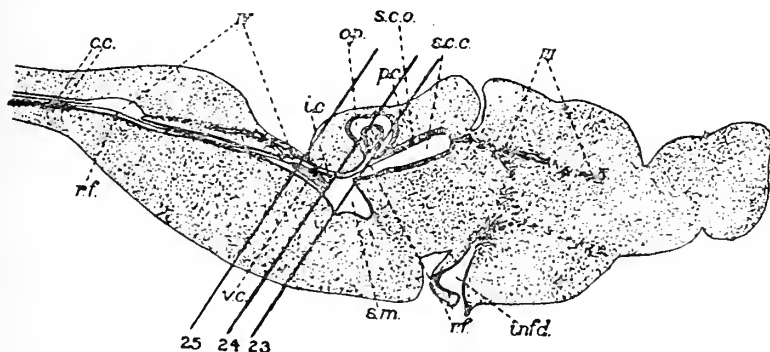
The fourth ventricle, too, is enormously reduced, owing, as I believe, to the fusion of its walls, mesially, at the level of about half the height of the ventricle, followed by the more or less complete obliteration of the upper chamber so cut off from the rest of the ventricle. Here, again, there is considerable variation, the vestiges of the upper portion of the ventricle being much more considerable in some specimens than in others.

The reduction of the ventricles has been least marked in the mid-brain, and the mesocœl forms the most considerable of the brain cavities. Postero-ventrally it is dilated into a large subspherical chamber, which I shall distinguish as

the sinus mesocœlicus (Text-fig. 7, *s. m.*). It continues antero-dorsally as a short wide tube which divides very soon into two canals, one passing anteriorly (figs. 21, 22, and Text-fig. 7, *s. c. c.*), and the other almost directly dorsally (figs. 21, 22, and Text-fig. 7, *op.*).

Of these, that which extends anteriorly is quite constant in its relations. In all my specimens it runs forwards to a point slightly postero-ventral to the habenular ganglia. In one case only was it seen actually to communicate in front with a remnant of the third ventricle. In the others it ended

TEXT-FIG. 7.



A slightly diagrammatic median sagittal section through the brain of *Bdellostoma* (*Polistotrema*) *stouti*. (The lines 23, 24, 25 indicate roughly the levels at which the sections represented in Pl. 3, figs. 23, 24, and 25 are taken. The sections are not, however, nearly so obliquely cut as would appear, for the specimen which was cut transversely was one in which the brain cavities were much more extensive, and in which, therefore, it was possible for the various cavities to appear in sections which were much more nearly transverse.) *c.c.* Canalis centralis. *i.c.* Isthmic canal. *inf.d.* Infundibular cavity. *op.* Optocœl. *p.c.* Posterior commissure. *r.f.* Reissner's fibre. *s.c.c.* Sub-commissural canal. *s.c.o.* Sub-commissural organ. *s.m.* Sinus mesocœlicus. *v.c.* Ventricular canal. *III.* Third ventricle. *IV.* Fourth ventricle.

blindly, the third ventricle being in this region wholly obliterated, and represented merely by the scattered nuclei of its vestigial ependymal epithelium.

In transverse sections (fig. 23, *s. c. c.*, which should be

compared with figs. 21, 22, and Text-fig. 7), this anterior portion of the mid-brain ventricle has a practically circular outline. Its diameter for the greater part of its length remains nearly constant, but increases slightly near the junction, posteriorly, with the second and more dorsally directed canal.

In that portion of the brain which immediately overlies the anterior extension of the mesocœl there is a commissural tract which has been identified by Edinger ('06) and Sterzi ('07) as the posterior commissure, to which conclusion my own observations have also led me. The anterior canal or chamber itself is not, however, I believe, the homologue of the whole of the iter in this region, the greater part of that passage beneath the posterior commissure having been obliterated.

The portion which persists (figs. 21-23 and Text-fig. 7, s. c. c.) is completely invested by an extraordinarily developed high columnar epithelium, from which the delicate fibrillæ of Reissner's fibre spring. This epithelium, therefore, represents the sub-commissural organ, and the enclosed space is almost certainly nothing but the product of the complete concurrence of a pair of ependymal grooves. I shall hereafter speak of this canal as the "sub-commissural canal."

I have pointed out above that the two halves of the sub-commissural organ, primarily distinct (e. g. *Petromyzon fluviatilis*), have in *Geotria* united beneath the anterior portion of the posterior commissure to form a median structure, which appears, in transverse section, as a horse-shoe-shaped band.

This tendency towards fusion in the middle line dorsally, seen in *Geotria* alone amongst the *Petromyzontidæ*,<sup>1</sup> has become the rule in higher vertebrates. Not only so, but, since in these higher forms the iter becomes compressed and narrowed from side to side (cf. figs. 4, 7), the ventral edges of the lips of these ependymal bands often nearly meet below (cf. figs. 2, 6). In such cases the iter may be more

<sup>1</sup> *Petromyzon planeri* (?), vide supra.



or less reduced to a tube partly enclosed by the sub-commissural organ. In Myxinoids an actual ventral fusion of the right and left halves of the sub-commissural organ has clearly taken place, and the two grooves have been merged in the single tubular sub-commissural canal. The lower portion of the iter, which existed originally ventral to the sub-commissural organ, has been obliterated. In one of my specimens, however, a trace of this lower portion has persisted as a small canal which runs forwards from the antero-dorsal region of the sinus mesocœlicus. Its course is parallel to, and but slightly ventral to, the sub-commissural canal. At first cylindrical, it gradually tapers away and is lost at a point considerably posterior to the anterior end of the sub-commissural canal. It is lined by ordinary columnar epithelium, and at its posterior end is separated from the sub-commissural organ by but a slight thickness of nervous tissue.

It was in this same specimen, too, that the third ventricle was least reduced, a small canal lined by flattened epithelium continuing forward the lumen of the sub-commissural canal for some distance into the 'tween brain. A small isolated chamber lying dorsal and anterior to the sub-commissural canal apparently represents a reduced infra-pineal or a diacœlic recess.

The second division (figs. 21-23, Text-fig. 7, *op.*) of the short wide canal leading from the sinus mesocœlicus, which I have spoken of as more dorsally directed than the sub-commissural canal, is apparently much more variable. The epithelium, too, which lines it, is, over the greater part of its extent, much less developed, and is markedly elongated only in the region immediately adjacent to its junction with the sub-commissural canal. There can be little doubt that this dorsal or postero-dorsal canal in the upper portion of the mid-brain represents the cavity of the optic lobes of the higher vertebrates, and is equivalent, therefore, to that large space which, in the Petromyzontidæ, lies above and behind the posterior commissure. In the Myxinoidei, however, the

roof in fore-, mid- and hind-brain brain has developed a nervous layer over its whole extent which may become exceedingly thick. At the same time the thickening of the walls of the brain has proceeded to such an extent that, as already stated, the third ventricle has been almost wholly, and the iter largely, obliterated.

In the region of the optic lobes it appears probable that the thickening must have first reduced the optocœl to a flattened circular or discoidal space. The apposition of the opposite walls over a considerable area in the middle of this space must have followed. In the result the optocœl has become a more or less annular cavity. Several stages in the reduction of the optocœl may be observed in the brains of my specimens of *Bdellostoma*.

In the specimen, already referred to, in which the ventricles have been least reduced, the optocœl is seen to have retained this almost perfectly annular shape. This same condition was observed in a second series, which was cut slightly obliquely to the sagittal plane, and is represented slightly diagrammatically in Text-fig. 7, which was obtained by the superposition of camera drawings of several adjacent sections.

In a third specimen (fig. 21) the posteriorly recurved portion of the optocœl no longer opens ventrally into the iter, and a further stage of reduction of this cavity is shown in the fourth specimen (fig. 22), where the hinder part of the optocœl has been altogether suppressed. The latter photomicrograph shows particularly well the highly developed epithelium (*s. c. o.*), which lines the sub-commissural canal (*s. c. c.*) and is continued into the optocœl upon its anterior wall, where it clothes the posterior surface of the posterior commissure.

In every case, however, there is a rapid transition from the extremely elongated cells of this epithelium into a much shorter columnar ependymal epithelium in those parts of the lumen remote from the posterior commissure.

The short common canal formed by the junction of the sub-commissural and optocœlic canals leads postero-ventrally into

the sinus mesocœlicus (Text-fig. 7, *s. m.*; figs. 21, 22, *s. m.*', *s. m.*''). This chamber, which, as already stated, forms by far the most considerable of the brain ventricles, must be regarded as equivalent to the hinder portion only of the iter of other vertebrates.

Sanders has described this chamber (in *Myxine*), as seen in sagittal section, as like a pipe-bowl in shape, and this description would apply equally well to the sinus mesocœlicus of *Bdellostoma*. In this latter animal, however, it is partly separated by a marked horizontal constriction into upper and lower chambers (figs. 21-23, *s. m.*', *s. m.*''). It is into the upper chamber that there opens the small anterior prolongation of the iter above referred to.

The horizontal constriction becomes more pronounced posteriorly, and finally forms a distinct horizontal partition which completely separates the two chambers (fig. 24). The two chambers so formed are at first of nearly equal size and are separated only by an epithelial partition. Further back the dividing lamella becomes much thicker and the two spaces become reduced in size and canal-like.

The upper one (fig. 24 and Text-fig. 7, *i. c.*), the posterior continuation of the upper chamber of the sinus mesocœlicus, is, without question, the homologue of the isthmus canal of the *Petromyzontidæ*, and must be considered as part of the mesocoel. It passes backwards with but slight alteration in size, but loses its circular outline (fig. 25, *i. c.*) and becomes flattened dorso-ventrally. Presently it opens widely, ventrally, into the fourth ventricle.

The lower canal (fig. 24 and Text-fig. 7, *v. c.*), which I have called the "ventricular canal," diminishes in size very rapidly, and, after separating somewhat widely from the isthmus canal, again approaches it (fig. 25), the two ultimately reuniting to constitute the fourth ventricle.

At the point of junction of these canals there is a slight dilatation (Text-fig. 7), but through the greater part of the extent of the medulla oblongata the fourth ventricle is chiefly represented by the narrow posterior continuation of

the ventricular canal. It is lined by a very ordinary short columnar epithelium.

In the thickened roof of the fourth ventricle there is, however, a chain of small, discontinuous and somewhat irregular spaces lined by a flattened epithelium, which continue backwards in the middle line directly dorsal to the fourth ventricle (Text-fig. 7). The most anterior of these spaces is, in one specimen, barely separated from the anterior dilated portion of the fourth ventricle by an epithelial wall.

Frequently these small spaces are divided vertically by a nearly median partition, and then appear as paired cavities lying on either side of the middle line. In one case there was a median space with a small lateral chamber on either side. It is clear that collectively they represent the vanishing vestiges of the dorsal portion of the fourth ventricle. I was able to make them out most satisfactorily in a series of sections cut transversely. In other series, cut sagittally, they are much less conspicuous.

In every brain examined, however, there was to be observed towards the hinder end of the medulla oblongata, at the level of these vestigial ventricular spaces (and therefore well above the continuous tubular portion of the fourth ventricle), a considerable space. This, which is to be regarded merely as an enlarged member of the dorsal chain of small spaces, is in wide communication below with the fourth ventricle in one specimen only (Text-fig. 7). In the three remaining specimens it is separated from the fourth ventricle by a thin layer of epithelium. Just behind the communication between the two cavities (where such communication occurs) the upper space tapers off into a narrow canal, which runs backwards dorsal and parallel to a similar backward continuation of the ventricular canal, the two giving rise to the double canal so characteristic of the spinal cord of the Myxinoidea. I look upon the dilatation of the last member of the upper discontinuous series of spaces as indicating approximately the hinder end of the medulla oblongata.

Reissner's fibre arises at the forward extremity of the mid-brain in a very large number of exceedingly fine fibrillæ from the cells of the sub-commissural organ surrounding the sub-commissural canal. These join together to form fine threads, which, in one specimen, are definitely seen as a pair of larger factors. These are situated nearly centrally in the sub-commissural canal, and become stouter as they pass backwards from their brush-like origin. At a point near the middle of the length of the sub-commissural canal the lesser fibres join up into the very definite fibre of Reissner, the diameter of which is seen to increase markedly as it is followed backwards.

In the one series cut transversely the fibre could not be followed. In another series (cut sagittally) it has sprung forwards from the *canalis centralis*, to lie, as a thickened rod, in the dorso-posterior portion of the *sinus mesocœlicus*. In both of the remaining specimens it has preserved its normal position, and may be observed as a tautly stretched thread (fig. 22, *r.f.*) passing from the sub-commissural canal through the upper chamber of the *sinus mesocœlicus* and so into the isthmic canal. From this it emerges into the fourth ventricle and passes backwards along the lower of the two divisions of the *canalis centralis* of the spinal cord (figs. 31, 32, *r.f.*). In the section photographed for fig. 21 a considerable length of Reissner's fibre occurs in the isthmic canal, but the magnification is too small to render the fibre visible.

The sub-commissural organ, owing to the complete fusion of its two halves ventrally as well as dorsally, has assumed the shape of a test-tube with its sealed end forward (the sub-commissural canal, figs. 21-23, and Text-fig. 7, *s.c.c.*). It slopes backwards and downwards beneath the posterior commissure, which is very ill defined and which appears to extend through nearly half of the length of the mid-brain. Behind the posterior commissure the upper half of the sub-commissural organ bends dorsally into the *optocœl*, where it passes gradually into a more ordinary columnar epithelium.

Ventrally it ends abruptly, being quite sharply marked off from the epithelium of the sinus mesocœlicus.

The actual cells of the sub-commissural organ are, like those of the Petromyzontidæ, extremely elongated and fibre-like. In both *Bdellostoma* and *Myxine* they attain a length of about  $50\ \mu$ , or approximately half of the diameter of the sub-commissural canal.

The fibrillæ of Reissner's fibre spring most freely from the anterior portion of the organ, but some continue to join the fibre along the whole extent of the sub-commissural canal. I have not been able to detect any fibrillæ arising from the epithelium which passes into the optocœl, though it is not improbable that some strands may actually have their origin there. In one specimen, indeed, a very fine factor appeared to issue from the optocœl to join Reissner's fibre.

#### *Bdellostoma cirrhatum.*

In the single series of sections through the brain of this species Reissner's fibre can only be seen in some sections. The sections were transverse and very thick, and the fibre had doubtless fallen away (it being in thick transverse sections difficult to attach the sections of fibre sufficiently to the slide). The ventricles of the brain very closely resemble the condition described as occurring in that specimen of *B. stouti* in which they had been least reduced. The cavity in the upper part of the hind-brain is relatively spacious and in open communication with the fourth ventricle. In the mid-brain there is a quantity of coagulum which, both in the sinus mesocœlicus and in the ventricular canal, might be mistaken for tangled and netted masses of Reissner's fibre. It was probably some such condition as this which misled Ayers (see *infra*).

#### *Myxine glutinosa.*

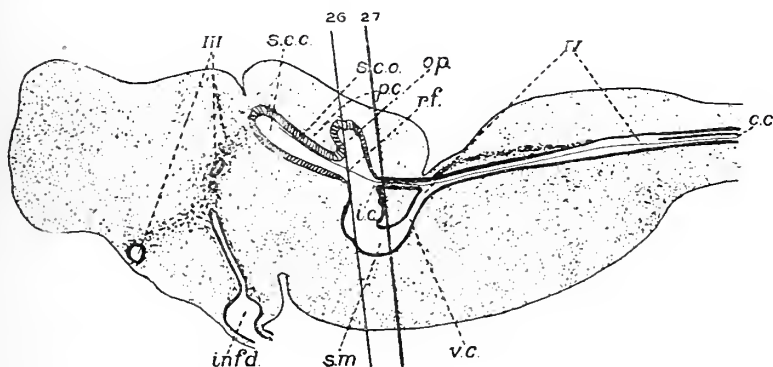
Of this species all but two of my specimens had been preserved entire in alcohol. As spirit usually penetrates



much more rapidly than does formalin I was rather surprised to find that while the formalin-preserved material gave me quite good results the spirit material was much less satisfactory. I can only suppose that this latter had been dead for some time before preservation was attempted.

Text-fig. 8, which, like the corresponding figure of *Bdellostoma*, was obtained by superposing camera drawings of several adjacent sections, represents diagrammatically the condition of the brain of *Myxine* as seen in sagittal section, and the course of Reissner's fibre through the ventricles.

TEXT-FIG. 8.



A diagrammatic median sagittal section through the brain of *Myxine glutinosa*. (The lines 26, 27 indicate approximately the planes of the transverse sections reproduced in Figs. 26 and 27.) *c.c.* Canalis centralis. *i.c.* Isthmic canal. *infd.* Infundibular cavity. *op.* Optocel. *p.c.* Posterior commissure. *r.f.* Reissner's fibre. *s.c.c.* Sub-commissural canal. *s.c.o.* Sub-commissural organ. *sm.* Sinus mesocælicus. *v.c.* Ventricular canal. *III.* Third ventricle. *IV.* Fourth ventricle.

Like that of *Bdellostoma*, the brain of *Myxine* is exceedingly variable, but in none of the three brains examined has there remained any trace of an open connection between the ventricles of the fore- and mid-brain.

In the mesocæl in every case five distinct chambers could be recognised. A well-marked sub-commissural canal (*s. c. c.*), completely invested by the highly specialised epi-



thelium of the sub-commissural organ (*s. c. o.*), and an optocœl (*op.*), which appears as a short bluntly ending and dorsally directed canal much more reduced than the corresponding canal in *Bdellostoma*, both open postero-ventrally into the sinus mesocœlicus (*s. m.*). From this, two canals, isthmie (*i. c.*) and ventricular (*v. c.*), lead backwards, but whereas in *Bdellostoma* it is the isthmie canal which is much the larger, in *Myxine* the ventricular canal is large and the isthmie canal has become a very narrow channel (compare figs. 25 and 27).

The latter opens widely into the upper portion of the sinus mesocœlicus on the level of the posterior end of the sub-commissural canal, but diminishes rapidly (funnel-wise) and extends through the greater part of the thickness of the postero-ventral portion of the optic lobes as a very fine canal, nearly oval in transverse section and comparatively remote from the backwardly-bulging sinus mesocœlicus.

The sinus mesocœlicus at its lower end is somewhat constricted and passes into the ventricular canal, which curves upwards and backwards to meet the isthmie canal as it makes its exit from the mid-brain. The junction of the two canals marks the beginning of the fourth ventricle, which, as in *Bdellostoma*, comes, at this point, more nearly to the dorsal surface of the brain than elsewhere. In *Myxine*, indeed, it is separated from the vascular tissue which everywhere envelops the brain by a thin layer of epithelial tissue only.

Posteriorly the roof of the hind brain thickens considerably, and again as in *Bdellostoma*, a series of small irregular chambers are to be made out beginning from a point immediately behind the posterior end of the isthmie canal. In some specimens they actually begin on either side of the isthmie canal, at its point of junction with the ventricular canal, as a pair of small chambers. Thence they extend in the middle line backwards to a point near the middle of the length of the medulla oblongata. Beneath this series of small chambers the fourth ventricle passes backwards, changing

in shape, as seen in transverse sections, through triangular to oval and oblong. Finally, it becomes circular, narrowing continually until it has a diameter scarcely greater than that of the *canalis centralis*, into which it presently passes.

At that point, however, where the upper discontinuous remnants of the fourth ventricle cease, I found in two of my specimens a considerable space (fig. 30, *x.*), which appears to be without epithelial lining. It is, nevertheless, lined, I believe, by an extremely flattened epithelium, the sparse nuclei of which are dotted at irregular intervals upon its surface. In one of the two specimens in which the cavity is present, greatly attenuated epithelial cells separate the chamber from the underlying fourth ventricle. In the other example the two cavities are in open communication.

At the posterior end of the medulla the fourth ventricle passes into a double *canalis centralis* (text-fig. 8, *c. c.*), but whereas in *Bdellostoma* (fig. 31) the upper canal is considerably the larger, in *Myxine* (fig. 28) the two canals are of much the same size.

Reissner's fibre (text-fig. 8, *r. f.*) has a course practically identical with that of the fibre in *Bdellostoma*. It arises in precisely the same way from a brush-like mass of delicate fibrillæ near the anterior end of the sub-commissural organ. These, however, appear to unite into a single thread which lies closely against the epithelium of the sub-commissural organ (fig. 26, *r. f.*). It traverses the upper part of the *sinus mesocœlicus*, and passes into the isthmus canal, lying closely against the upper wall of that passage (fig. 27, *r. f.*). Emerging from the isthmus canal it extends backwards through the fourth ventricle (fig. 30, *r. f.*), and where that gives place to the double canal of the spinal cord it passes into the lower of the two canals (figs. 28, 29, *r. f.*), exactly as in *Bdellostoma*.

It apparently extends through this lower canal practically to the extreme posterior end of the body. Slightly in front of the actual extremity, however, the two canals reunite to form a single canal which appears in transverse section as a

rather narrow vertical cleft. The spinal cord itself turns downwards behind the end of the notochord and becomes enlarged to partly enclose the large sinus terminalis. I was able to make out this condition in but one of the three tails examined in sagittal section; the other two had become twisted during the preparation of the material for sectioning, and in consequence were cut so obliquely as to be unintelligible.

Both Sanders and Studnička seem also to have found such a terminal sinus, and there appears to be no reason to doubt that this is the normal condition of the end of the spinal cord.

In the one specimen examined, the walls of the sinus are clothed antero-dorsally by the ependymal epithelium of the *canalis centralis*, but posteriorly the space is enclosed only by the connective tissues of the meninges. We have thus in *Myxine* a sinus terminalis into which the *canalis centralis* opens by a terminal neural pore exactly as in the *Petromyzontidæ*.

Through this terminal neural pore Reissner's fibre passes, and, in the one specimen in which the relations of these parts could be clearly made out, ends in a large intricately coiled mass, which Sanders has aptly described as a "a mulberry-like mass of glass-like aspect."

A central section through the mass is shown in fig. 18, which is reproduced from an actual photomicrograph. The sections had been stained simply with borax-carmin in bulk, and the mass of fibre was only faintly tinged with pink. After the photomicrograph had been taken the sections were double-stained with picro-indigo-carmin. The coiled terminal mass of fibre became stained green or blue-green in a manner absolutely unlike that in which a nerve-fibre stains. Another photomicrograph was taken, this time of a section nearer to the surface of the mass, and is reproduced as fig. 17. This shows some loose coils of Reissner's fibre lying near the apex of the mass, and close to the opening of the terminal neural pore.

The photomicrographs are not nearly as perfect as I could

wish, and do not represent at all adequately this wonderful mass of fibre; it was, however, a particularly difficult subject to photograph, both on account of the staining and also on account of the thickness of the sections ( $20\ \mu$ ).

Some idea of the relatively enormous size of this mass of coiled fibre may be formed when it is stated that it appears in four or five adjacent sagittal sections, each  $20\ \mu$  in thickness. It thus probably had a thickness of not less than  $80\ \mu$  from side to side. Measured dorsi-ventrally the mass has a length of close upon  $100\ \mu$ , while its average antero-posterior diameter is nearly  $80\ \mu$ .

The *canalis centralis* a little forward of the *sinus terminalis* is empty of fibre, and I estimate that the mulberry-like mass represents some 200 to 250 mm. of fibre retracted into a heap barely 0.1 mm. high. Since the specimen was little over a foot in length I can only suppose that the fibre must have snapped at a point very far forward. This breakage must have been followed by a continuous recoil of the hinder piece into the *sinus terminalis*, such recoil having been completed before fixation was effected.

Sagittal sections through the tail of a second specimen appeared to show a somewhat similar condition, but the sections were so crumpled and distorted in this region that it is not possible to speak with certainty.

Such a condition is, however, undoubtedly of frequent occurrence (Sanders '94, Studnička, '99), and must be the result of a breakage of the fibre at some time shortly before fixation. It is certainly not the condition of the functional fibre, which should be inserted, as in the *Petromyzontidæ*, as a tautly stretched thread, into the meningeal portion of the wall of the *sinus terminalis*. This normal condition has, so far as I can discover, been observed only by Sanders ('94, p. 11).

#### Critical Discussion.

As already noted, an account of the occurrence and relations of Reissner's fibre in *Myxine* was given by Sanders as

long ago as 1894. That author was the first to trace the fibre forwards into the mid-brain and backwards into the sinus terminalis, and he, too, was the first to point out that the canal centralis was widely open posteriorly (in *Myxine*), and that through this opening Reissner's fibre passed. Apparently he had formed no definite opinion as to the character of this "central rod," as he called it, in *Myxine*, although in an earlier paper he had accepted Stieda's dictum that it was merely an artifact.

His account of the splitting of Reissner's fibre in the fourth ventricle into two portions, one of which passes through the isthmic canal and the other through the lower canal (the ventricular canal of my descriptions), is, however, erroneous. It is true that in material that is not well preserved there is found a quantity of coagulum which presents the appearance of a network of fibres, and which might be mistaken for Reissner's fibre. In well-preserved material, however, this is absent, and the unmistakable fibre stretches tautly from its point of origin in the anterior part of the sub-commissural canal through the upper portion of the sinus mesocœlicus, and thence through the isthmic canal, nowhere receiving any conspicuous factor or giving off any important branch. The ventral portion of the sinus mesocœlicus and the ventricular canal are not in any of my specimens traversed by the fibre of Reissner. Indeed, I have suggested that the portion of the mesocœl which I have termed the isthmic canal has persisted in *Myxine* (when other portions of the brain-ventricles have been obliterated) because it is traversed by Reissner's fibre.

Studnička, whose findings, in other respects, confirm those of Sanders, says nothing of any part of the fibre passing elsewhere than through the isthmic canal.

The only other observations upon Reissner's fibre in *Myxinoids* are, I believe, those recorded by Ayers ('08). Although he does not refer to it by that name, but speaks merely of "ventricular fibres," I think that there can be no doubt that it is to Reissner's fibre that his work relates. He

speaks of the optocœl as the "cerebellar ventricle," and clearly regards the isthmic canal as part of the fourth ventricle, and claims to have found numerous branches of the fibre issuing from the "mid-brain ventricle" (the sub-commissural canal of my descriptions) and the "cerebellar ventricle" to join a main fibre. This main fibre was found only as a much coiled thread in the "fourth ventricle" (the sinus mesocœlicus and isthmic canal of my descriptions).

He speaks, moreover, of having examined the fibre "in section and by dissection" (my spaced type), which is rather a remarkable statement, for in none of my specimens does the fibre exceed  $2\mu$  in diameter, and it is visible only under quite considerable magnification. It is clear, however, from his description, that the fibre must have been broken or cut prior to the preservation of his material, and that a considerable length had retracted into the sinus mesocœlicus. Some loose loops, perhaps, may have been thrown into the optocœl and so account for the condition he describes. As already stated I found only in a single specimen (*B. stouti*) a very delicate branch coming from the optocœl and joining Reissner's fibre as it emerges from the sub-commissural canal.

The only real addition to our knowledge of Reissner's fibre made by Ayers is contained in his statement that the ultimate fibrillæ are derived from the cells of the ependymal epithelium. Unfortunately great importance cannot be attached to his account, for it is far from certain that much which he has interpreted as "ventricular fibre" is not really coagulum. In *Petromyzon*, both in larvæ and adults, he states that he was unable to find these fibres. In their place he found "a fine-meshed network of fibrils . . . which bears the same relations to the ependymal cells and in life practically fills the ventricular cavity." In this case there can be no doubt, from his description, that he refers to the fibre-like coagulum which commonly occurs in the brain-ventricles, and has altogether overlooked Reissner's fibre.

He suggests that the function of these fibrillæ is to bring



the ependymal epithelial cells into intimate connection, and that they are possibly in some way connected with the control of the lymph supply in the ventricles.

Holm makes no reference to the occurrence of Reissner's fibre, although he seems to have seen the isthmic canal, of which in *Myxine* it may be said that it has persisted merely because it gives free passage to that structure. In the case which he describes ('01, p. 369) the isthmic canal (his "upper canal") was evidently much less reduced than is commonly the case.

He has apparently followed Retzius ('94) in his erroneous identification of the ventricle of the mid-brain as the fourth ventricle. As I have pointed out above, the cavity of the mid-brain is greatly reduced, its anterior portion being represented only by the sub-commissural canal, while the whole of that large space (optocœl), which in the *Petromyzontidæ* (Text-fig. 5) lies above and behind the posterior commissure in the roof of the mid-brain, is reduced in *Bdellostoma* (Text-fig. 7) to a more or less complete annular space, and in *Myxine* (Text-fig. 8) to a short, dorsally directed and blindly ending canal. Behind, beneath the posterior portion of the corpora bigemina, the aqueductus Sylvii is, in the *Myxinoids*, reduced to two narrow passages, the isthmic and ventricular canals.

The main cavity, then, in the mid-brain ventricle, which, with the ventricular canal, is identified by Sanders,<sup>1</sup> Holm

<sup>1</sup> In justice to Sanders, whose work appears to have been largely overlooked, it should be pointed out that his observations were really remarkably correct, and that in many particulars he has anticipated the results of more recent workers. Not only did he identify correctly nearly all of the various brain-ventricles, but later work has also justified his identifications of various parts of the brain. He continued the observations of Retzius as to the absence of a cerebellum. Curiously enough, Holm ('01, p. 378) misrepresents Sanders as interpreting the corpora bigemina as the cerebellum, a mistake which, in view of Sanders actual statements, is altogether inexplicable. Thus ('94, p. 6), Sanders says, "It is remarkable that the cerebellum . . . is here entirely absent," and again (op. cit., p. 22), "*Myxine* appears to present



and Ayers as the fourth ventricle, represents actually but a small portion of the aqueductus Sylvii, and I have preferred, for that reason, to speak of it as the sinus mesocœlicus.

Sterzi ('07) identifies it correctly as the cavity of the mid-brain, he appears not to have noticed the isthmic canal in Myxine, although he saw and called attention (op. cit., p. 539) to the occurrence of small scattered spaces that continue backwards from it towards the upper canal of the spinal cord. He, so far as I can find, makes no reference whatever to the occurrence of Reissner's fibre.

Sargent ('04) quotes that part of Sanders' descriptions which relates to the course of Reissner's fibre in the brain, and dismisses it without further comment. He also quotes without comment the preceding paragraph, in which Sanders describes the ending of the fibre posteriorly, and, as above stated, he dismisses Studnička's statements which confirmed those of Sanders, remarking that the appearances so interpreted by Studnička must be due to the disturbed and abnormal condition of the fibre in his preparations.

Sargent's only other statement which bears upon the condition of the fibre in Myxinoids is the wholly unwarranted assumption ('04, p. 162) that "In Myxine, which is blind . . . Reissner's fibre must be made up wholly of axons from the olfactory centre in the ganglion habenulæ."

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the only instance in the vertebrate kingdom of the entire absence of an important section of the brain, viz. the cerebellum." It was Sanders, too, who first pointed out the existence of the terminal sinus in Myxine. Retzius to whom is commonly attributed the discovery of this terminal dilatation of the spinal cord, to which he gave the name of the terminal sinus, only published in the following year. Sanders' description of the condition of the hinder end of the canalis centralis of the spinal cord anticipated Sterzi's by more than a dozen years.

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## EXPLANATION OF PLATES 1-5,

Illustrating Prof. George E. Nicholls' paper on "The Structure and Development of Reissner's Fibre and the Sub-commissural Organ."—Part I.

## LIST OF REFERENCE LETTERS.

*c. bg.* Corpora bigemina. *c. c.* Canalis centralis of the spinal cord. *c. c.'* Upper canal of canalis centralis (in Myxinoids). *cil.* Cilia. *Dk.* Cells of the "Dachkern." *e. ep.* Ependymal epithelium. *ep.* Epiphysis. *fb., fb.'* Fibrillæ of Reissner's fibre. *h. g.* Habenular ganglion. *i. c.* Isthmic canal. *inf.* Recessus infrapinealis. *iter.* Iter. *mug.* Meninges. *m. r.* Mesocœlic recess. *m. t.* Cells of the terminal mass. *nch.* Notochord. *n. p. c.* Nucleus of the posterior commissure. *op.* Optocœl; optocœlic canal (Myxinoids). *op.'* Posterior extension of optocœlic canal. *p. c.* Posterior commissure. *r. d.* Dialectic recess. *r. f.* Reissner's fibre. *r. f.'* Reissner's fibre, tangled in canalis centralis. *r. f.{'* Reissner's fibre, tangled in sinus terminalis. *s. c.* Spinal cord. *s. c. c.* Sub-commissural canal. *s. c. o.* Sub-commissural organ. *s. m.* Sinus mesocœlicus. *s. m.'* Upper chamber of sinus mesocœlicus. *s. m.{'* Lower chamber of sinus mesocœlicus. *s. t.* Sinus terminalis. *t. c.* Tela choroidea II. *tect. mes.* Tectum mesencephali. *t. p.* Terminal plug. *v. c.* Ventricular canal. *x.* Large space (part of fourth ventricle) in roof of hind-brain, lying above the canal-like fourth ventricle. III. Third ventricle. IV. Fourth ventricle. \* Indicates the position of the extremity of the notochord in Fig. 52.

[Figs. 1-32 are all reproduced from photomicrographs; the remaining figures, 33-58, were drawn from the actual preparations with the aid of a camera lucida.]

## PLATE 1.

Figs. 1-9 represent the sub-commissural organ of typical members of the different vertebrate sub-classes, as seen in transverse sections of the brain.

Fig. 1.—*Petromyzon fluviatilis*. × 28.

Fig. 2.—*Raia blanda*. × 65.

Fig. 3.—*Scyllium canicula*. × 24.

Fig. 4.—*Esox lucius*.  $\times 28$ .

Fig. 5.—*Rana temporaria*.  $\times 128$ .

Fig. 6.—*Sphenodon* (Hatteria) *punctatus*.  $\times 24$ .

Fig. 7.—*Gallus domesticus* (19-day chick).  $\times 24$ .

Fig. 8.—*Microtus arvensis*.  $\times 52$ .

Fig. 9.—*Lepus cuniculus*.  $\times 24$ .

## PLATE 2.

Fig. 10.—*Petromyzon fluviatilis*. Part of a transverse section through the hinder part of the mid-brain, showing the paired condition of the isthmie canal.  $\times 320$ .

Fig. 11.—*P. fluviatilis*. Part of a horizontal section through the same region of the brain, showing the junction of the right and left factors of Reissner's fibre at a point just anterior to the isthmie canal (here median and not visibly a paired structure).  $\times 340$ .

Fig. 12.—*P. fluviatilis*. Part of a median sagittal section through the end of the spinal cord showing the *canalis centralis* expanding into a *sinus terminalis*, within which is seen a tangled mass of Reissner's fibre, lying against the meningeal wall.  $\times 250$ .

Fig. 13.—*P. fluviatilis*. A similar section (but slightly oblique) through the *sinus terminalis* of another specimen. Reissner's fibre is inserted into the meningeal sheath of the sinus.  $\times 250$ .

Fig. 14.—*Ichthyomyzon* (*Entosphenus*) *tridentatus*. A sagittal section through the tail of an ammocete of 105 mm. showing the *sinus terminalis* and Reissner's fibre.  $\times 80$ .

Fig. 15.—*I. tridentatus*. Part of a sagittal section through the tail of an ammocete of 90 mm. showing a considerable tangle of Reissner's fibre almost filling the *sinus terminalis*.  $\times 350$ .

Fig. 16.—*Petromyzon fluviatilis*. Part of a sagittal section through the spinal cord showing the interrupted coiling of Reissner's fibre.  $\times 600$ .

Fig. 17.—*Myxine glutinosa*.—Sagittal section through the *sinus terminalis*, showing a large mass of coiled Reissner's fibre. At the apex of the mass the fibre is more loosely looped.  $\times 260$ .

Fig. 18.—*M. glutinosa*. Another section from the same series as fig. 17, passing almost centrally through the mass of fibre.  $\times 260$ .

Fig. 19.—*Geotria australis*. Part of a median sagittal section through the posterior commissure, showing a tangle of Reissner's fibre below the sub-commissural organ (from one of Prof. Dendy's preparations).  $\times 310$ .



Fig. 20.—*G. australis*. Part of a median sagittal section through the hind-brain (from one of Prof. Dendy's preparations). A great length of Reissner's fibre is seen lying in the fourth ventricle and canalis centralis (somewhat displaced).  $\times 150$ .

## PLATE 3.

Fig. 21.—*Bdellostoma (Polistotrema) stouti*. Part of a median sagittal section through the mid-brain.  $\times 50$ .

Fig. 22.—*B. stouti*. Part of a nearly median sagittal section through the mid-brain of another specimen, showing the optocœl much more reduced. Reissner's fibre is seen issuing from the sub-commissural canal.  $\times 60$ .

Figs. 23–25.—*B. stouti*. Portions of transverse sections through the mid-brain of another specimen. These are taken approximately at the levels indicated by the lines 23, 24, 25 in Text-fig. 7.  $\times 68$ .

Figs. 26, 27.—*Myxine glutinosa*. Portions of transverse sections through the mid-brain, corresponding roughly to those shown for *Bdellostoma* in figs. 23, 25. The cavities in *Myxine* are, however, relatively much smaller. Fig. 26  $\times 115$ . Fig. 27  $\times 200$ .

Fig. 28.—*M. glutinosa*. Part of a transverse section through the spinal cord, showing the double character of the canalis centralis and Reissner's fibre in the lower of the two canals.  $\times 350$ .

Fig. 29.—*M. glutinosa*. Part of a sagittal section through the spinal cord.  $\times 60$ .

Fig. 30.—*M. glutinosa*.—Part of a sagittal section through the hind-brain and spinal cord, showing the large space (*x*) situated in the hind-brain above the canal-like portion of the fourth ventricle.  $\times 150$ .

Fig. 31.—*Bdellostoma stonti*. Part of a transverse section through the spinal cord for comparison with that of *Myxine* (fig. 28).  $\times 450$ .

Fig. 32.—*B. stouti*. Part of a sagittal section through the spinal cord showing Reissner's fibre in the lower canal.  $\times 68$ .

## PLATE 4.

Fig. 33.—*Petromyzon fluviatilis*. Part of a transverse section through the thalamencephalon, showing the sub-commissural organ (on the true left side) between the right habenular ganglion and the left optic thalamus.  $\times 30$ .

Fig. 34.—*P. fluviatilis*. Part of another transverse section, taken at a point immediately anterior to the posterior commissure.  $\times 30$ .

Fig. 35.—*P. fluviatilis*. Part of another transverse section, through the posterior commissure, showing the right diacœlic recess.  $\times 30$ .

Fig. 36.—*P. fluviatilis*. Another transverse section through the mid-brain, immediately behind the posterior commissure, showing the end of the sub-commissural organ.  $\times 30$ .

Fig. 37.—*P. fluviatilis*. Horizontal section through the sub-commissural organ, showing the paired character of Reissner's fibre. (Some of the lesser branches were added from adjacent sections.)  $\times 240$ .

Fig. 38.—*P. fluviatilis*.—Part of a transverse section through the posterior commissure, showing the sub-commissural organ and Reissner's fibre, cut obliquely, lying closely against it on each side.  $\times 240$ .

Fig. 39.—*P. fluviatilis*. Part of a horizontal section through the sub-commissural organ anterior to the posterior commissure, showing the fibrillæ of Reissner's fibre arising from the surface of the organ.  $\times 525$ .

Fig. 40.—*P. fluviatilis*. Median section through the extremity of the filum terminale, showing the canalis centralis opening widely by the terminal neural pore in the sinus terminalis. (The figure was obtained by the superposition of drawings of several adjacent sections which were cut slightly obliquely to the sagittal plane.)  $\times 160$ .

Fig. 41.—*Ichthyomyzon (Entosphenus) tridentatus*. A transverse section through the developing sub-commissural organ of an ammocœte 12 mm. long. (The level of the section is indicated by the line 41 in fig. 45.)  $\times 560$ .

Fig. 42.—*I. tridentatus*. A transverse section through the sub-commissural organ of an ammocœte 40 mm. long. The level of the section is approximately indicated by the line 42 in fig. 46.)  $\times 320$ .

Fig. 43.—*I. tridentatus*. A transverse section through the sub-commissural organ of an ammocœte 95 mm. long.  $\times 300$ .

Fig. 44.—*I. tridentatus*.—Part of a transverse section through the hinder part of the mid-brain of an ammocœte, 65 mm. long, showing the paired character of the isthmic canal.

#### PLATE 5.

Fig. 45.—*Ichthyomyzon tridentatus*. Part of a sagittal section through the roof of the brain of an ammocœte 145 mm. long, showing the extent of the posterior commissure and the sub-commissural organ.  $\times 525$ .

Fig. 46.—*I. tridentatus*. Part of a sagittal section through the roof of the brain of an ammocete 30 mm. long.  $\times 320$ .

Fig. 47.—*I. tridentatus*. Transverse section through the tail of an ammocete 12 mm. long, showing the condition of the *canalis centralis*.  $\times 420$ .

Fig. 48.—*I. tridentatus*. A similar section through the tail of the same ammocete, taken at a point 40 micra behind that represented in fig. 47.  $\times 420$ .

Fig. 49.—*I. tridentatus*. Another section from the same series, taken at a point 50 micra posterior to that shown in fig. 48, and distant only 20 micra from the actual end of the tail. It shows the *canalis centralis* widened out into a *sinus terminalis*.  $\times 420$ .

Fig. 50.—*I. tridentatus*. A median sagittal section through the tail of an ammocete 14.5 mm. long, showing Reissner's fibre ending in a terminal plug in the *sinus terminalis*.  $\times 518$ .

Fig. 51.—*I. tridentatus*. Median sagittal section through the tail of an ammocete 42 mm. long, showing Reissner's fibre expanded into a terminal plug inserted into the wall of the *sinus terminalis*.  $\times 394$ .

Fig. 52.—*I. tridentatus*. A similar sagittal section through the end of the tail of an ammocete 75 mm. long, showing Reissner's fibre coiled in the *sinus terminalis*.  $\times 394$ .

Fig. 53.—*I. tridentatus*. A section cut somewhat obliquely to the sagittal plane, showing Reissner's fibre ending in a terminal plug in the *sinus terminalis* of an ammocete 36 mm. long.  $\times 690$ .

Fig. 54.—*I. tridentatus*. A sagittal section through the tail of an ammocete 105 mm. long. (Reissner's fibre has been completed from one or two adjoining sections.)  $\times 94$ .

Fig. 55.—*I. tridentatus*. A nearly transverse section through the anterior part of the *sinus terminalis* of an ammocete 65 mm. long. Reissner's fibre has retracted into a coil, and some fibrous tissue appears to have been pulled into the *sinus terminalis*.  $\times 560$ .

Fig. 56.—*I. tridentatus*. A sagittal section through part of the spinal cord of an ammocete 34 mm. long.  $\times 560$ .

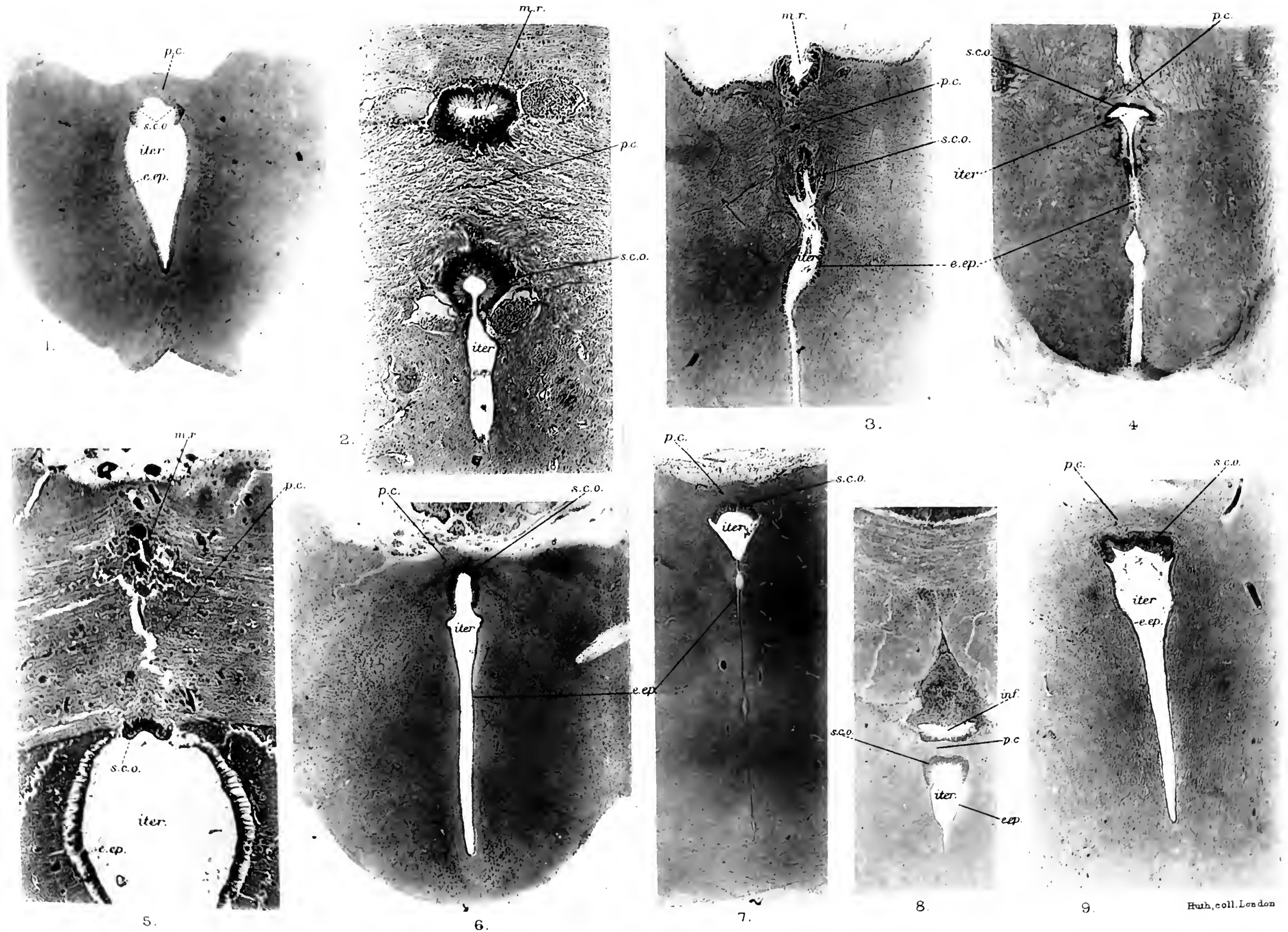
Fig. 57.—*Geotria australis*. Part of a sagittal section through the mid-brain of a velasia stage showing the pair of principal factors of Reissner's fibre uniting about halfway between the sub-commissural organ and the isthmus canal.  $\times 105$ .

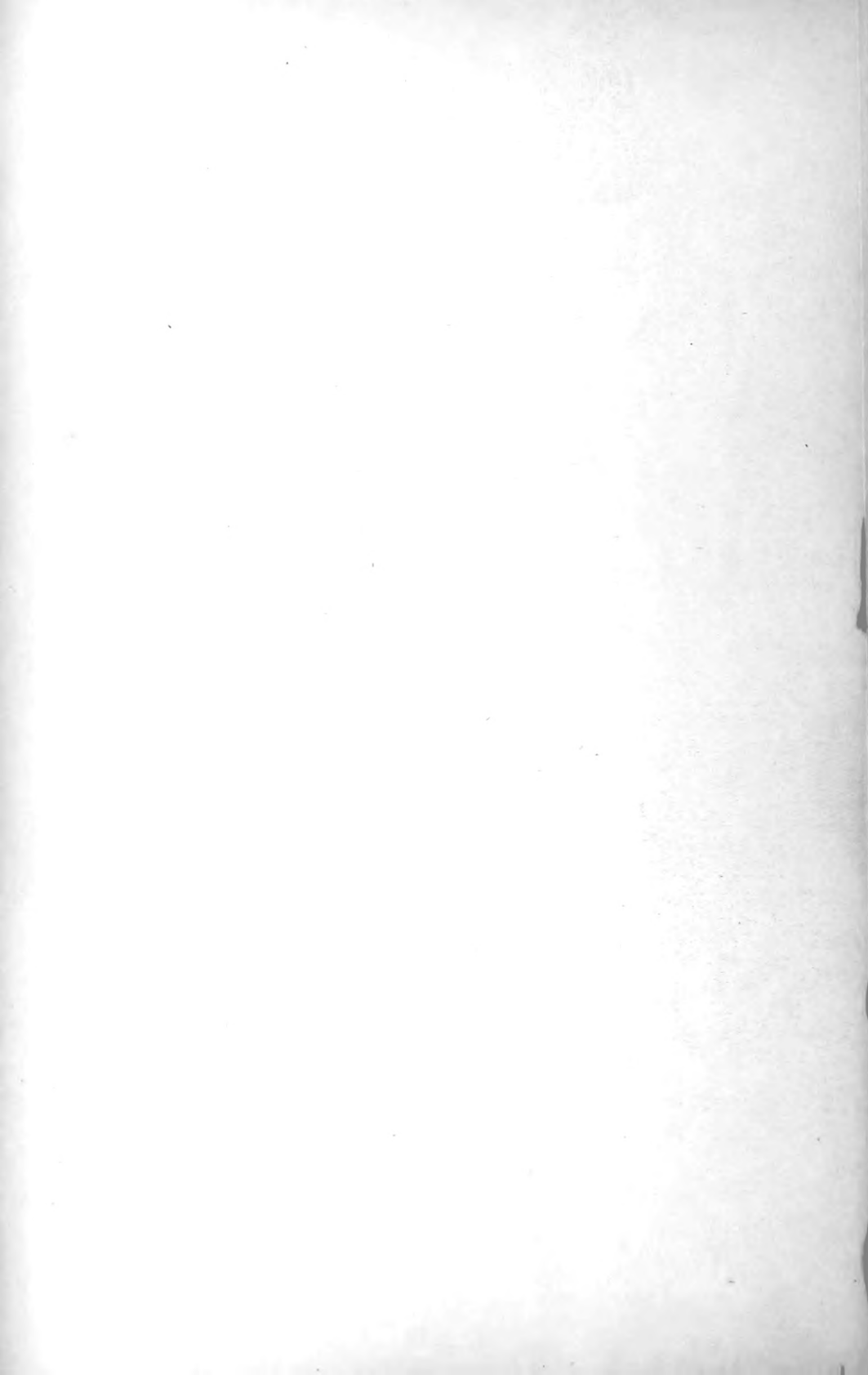
Fig. 58.—*G. australis*. A sagittal section through the posterior commissure of another velasia, showing Reissner's fibre broken and retracted slightly forwards. (From one of Prof. Dendy's preparations.)  $\times 104$ .







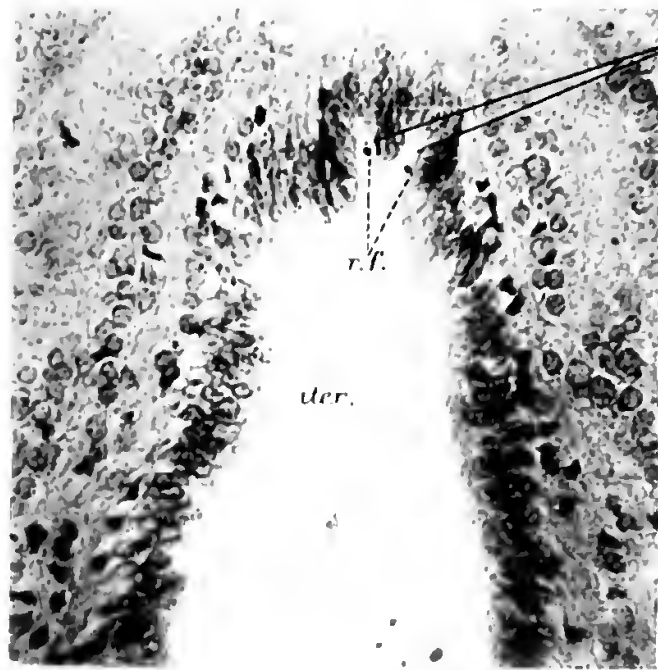




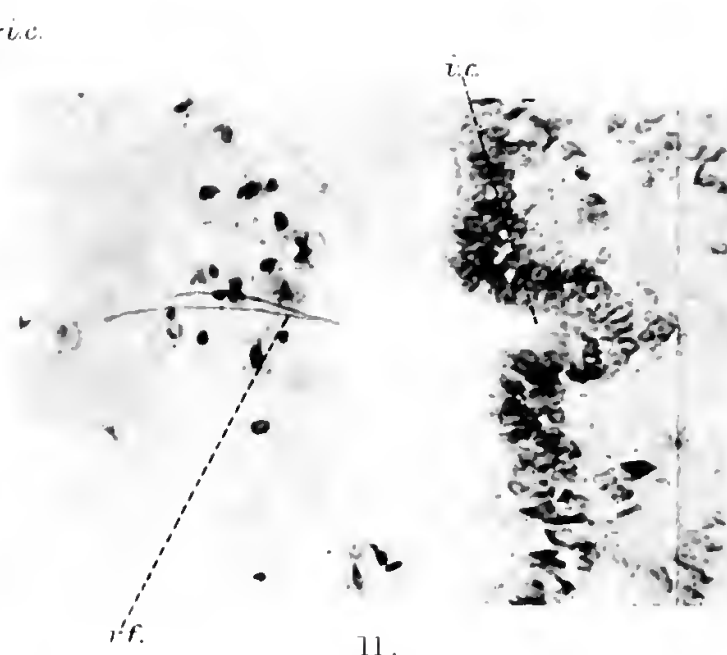




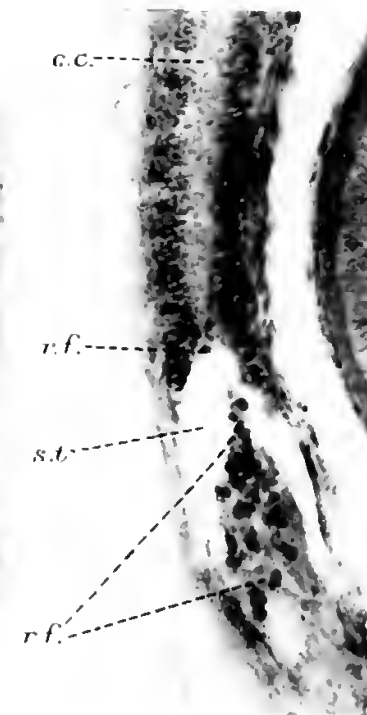




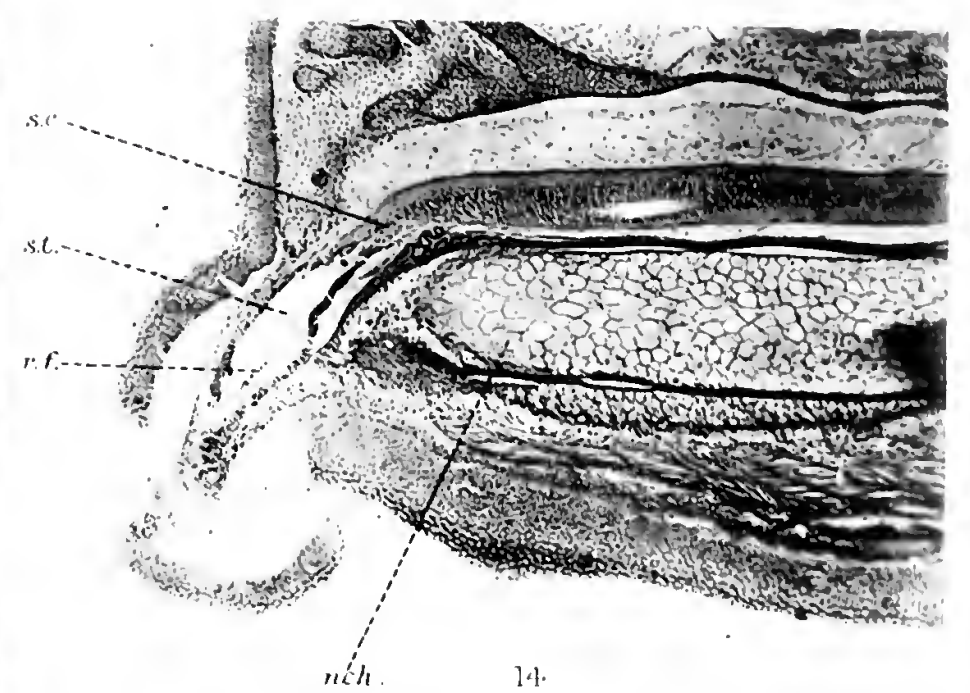
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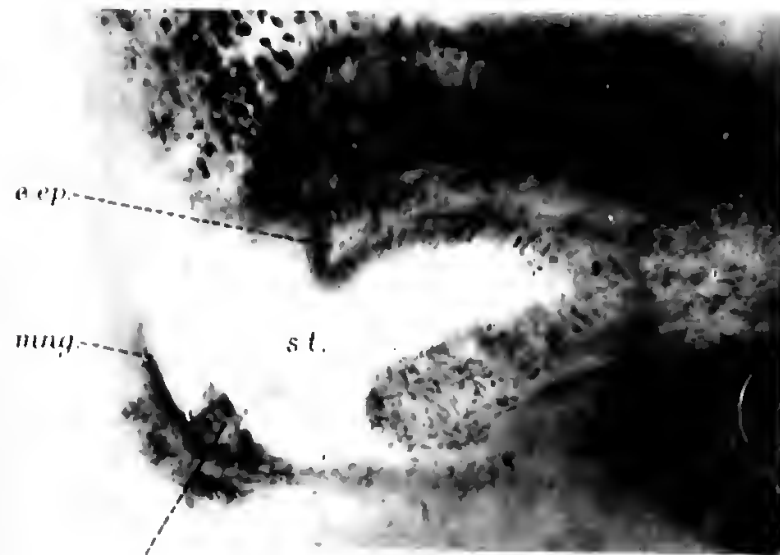
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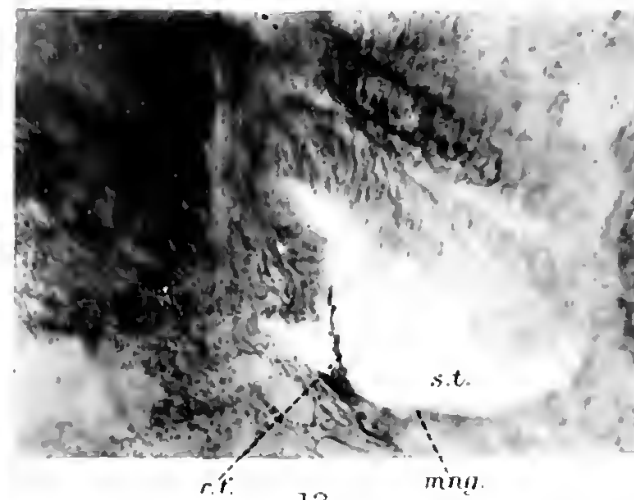
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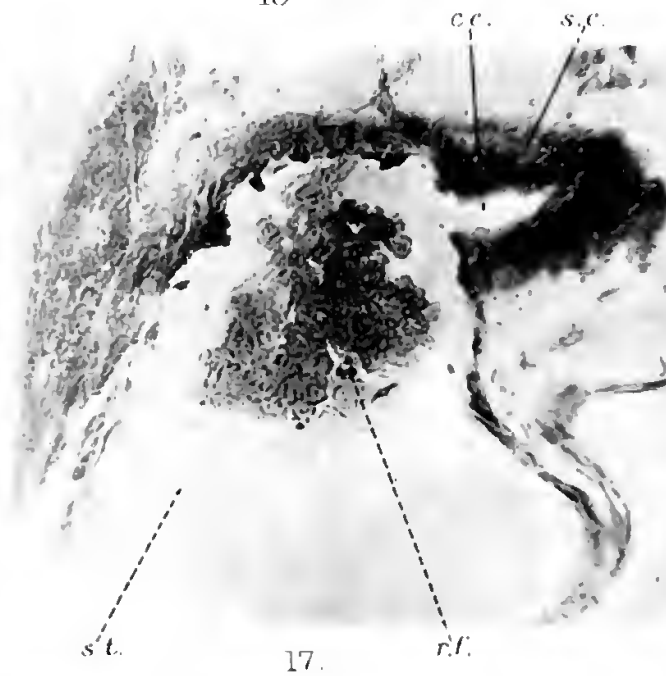
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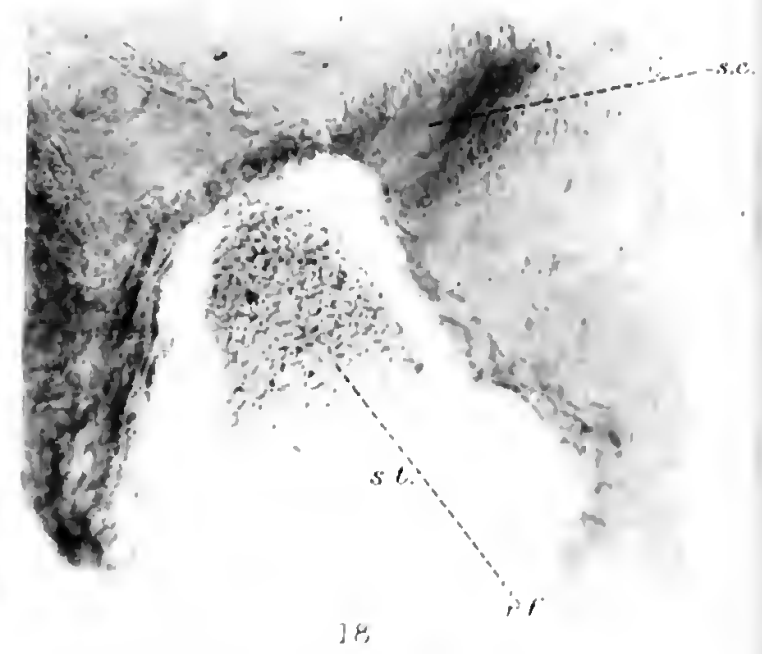
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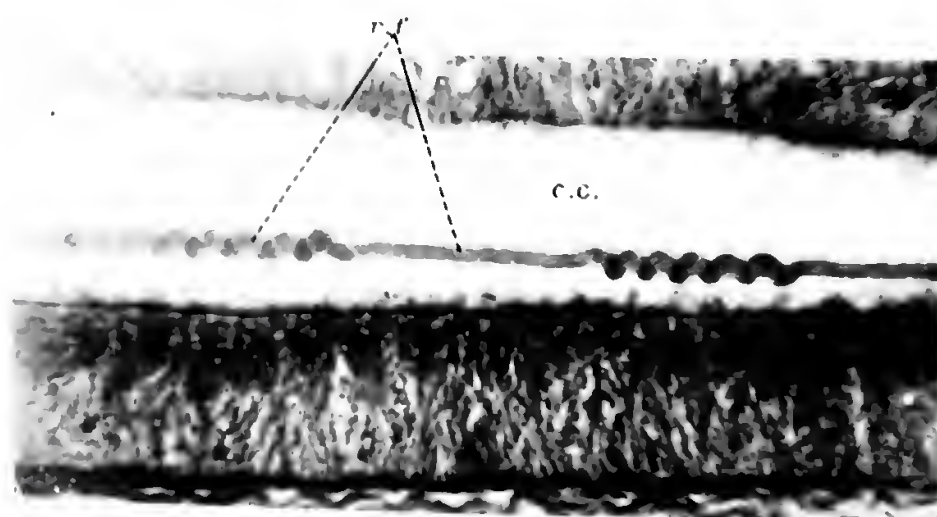
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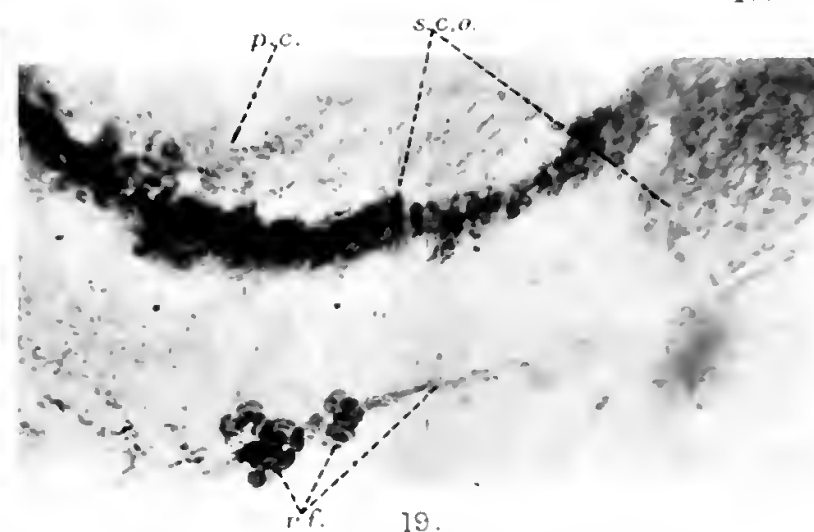
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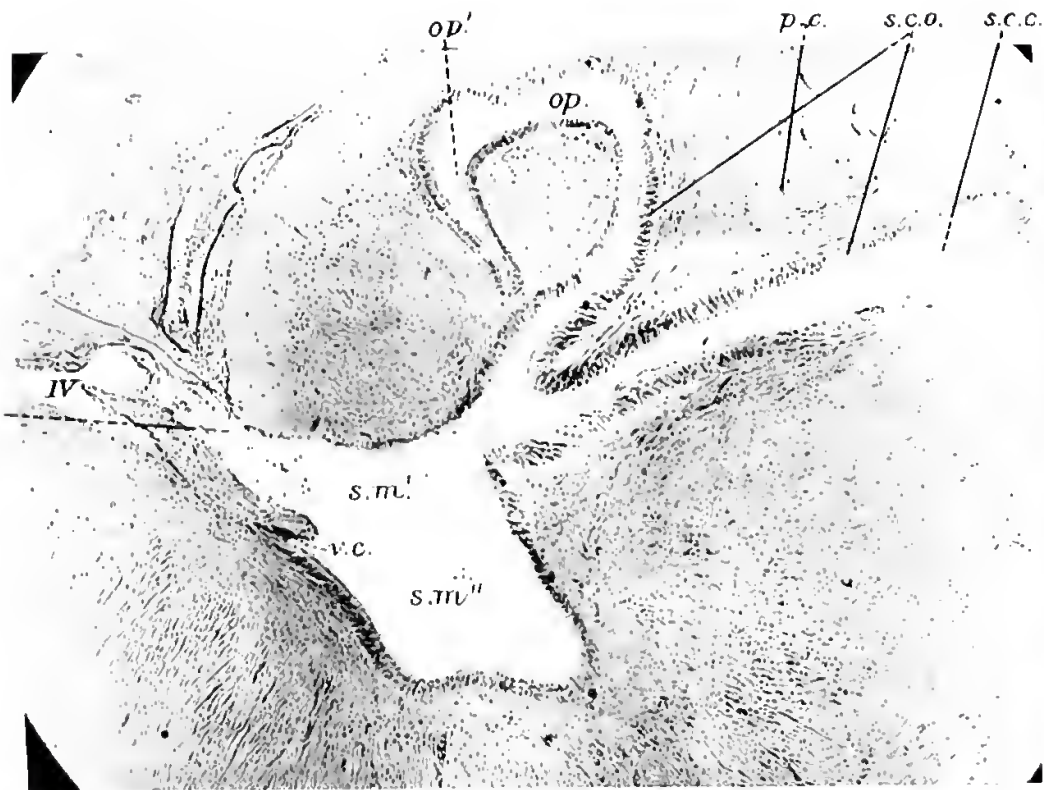
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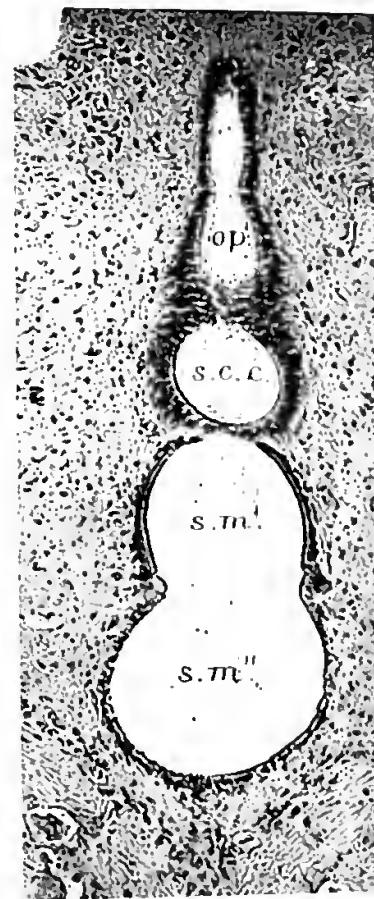




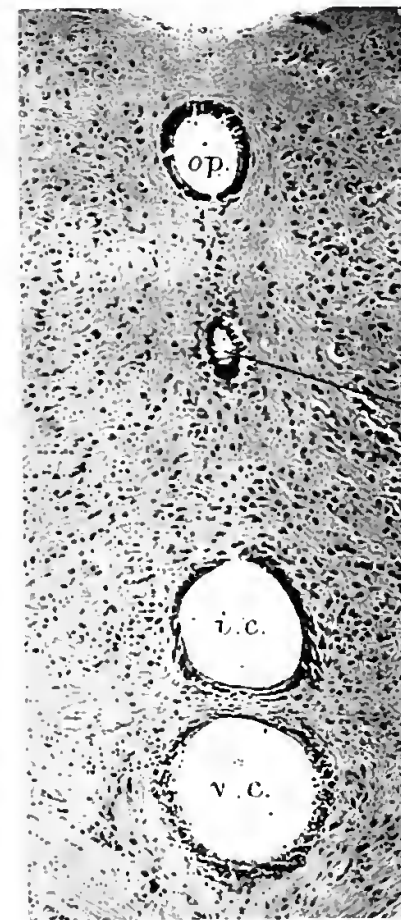




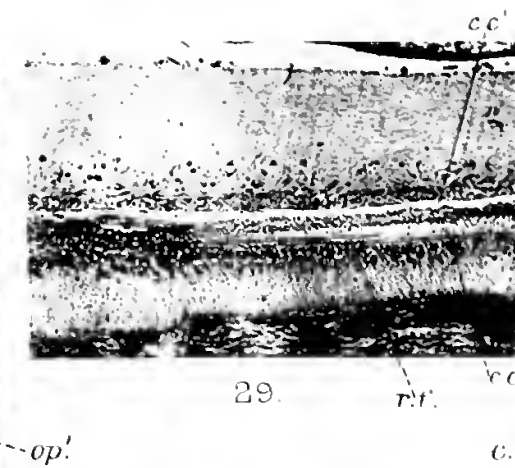
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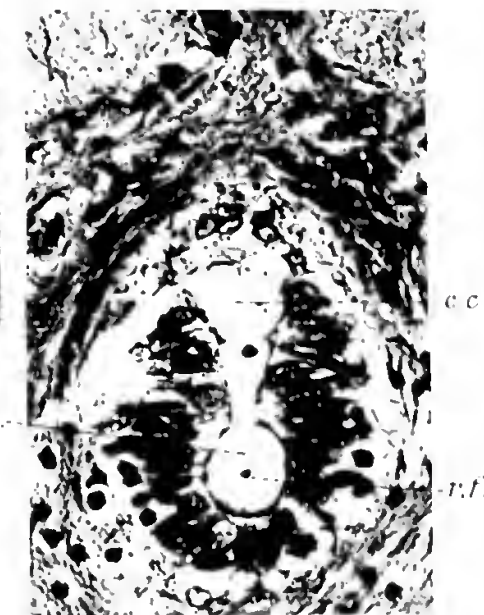
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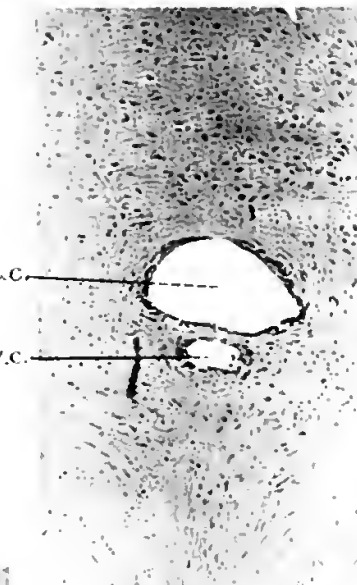
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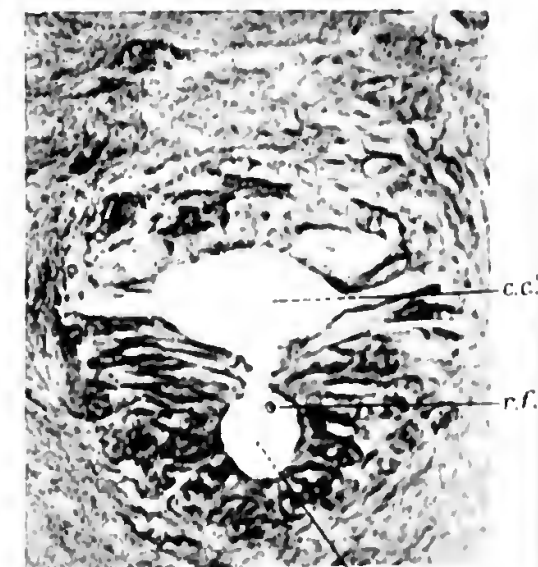
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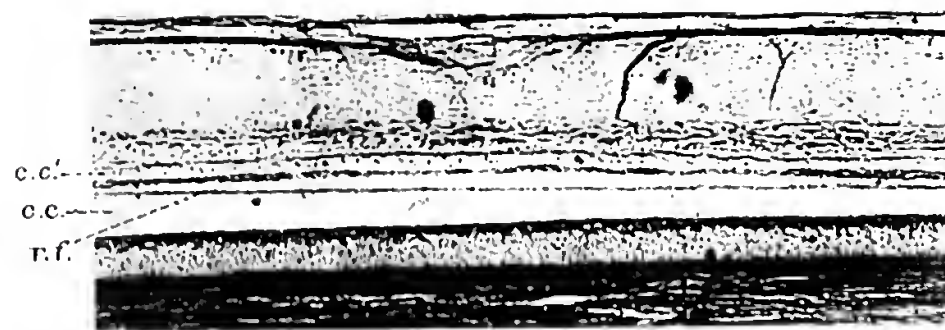
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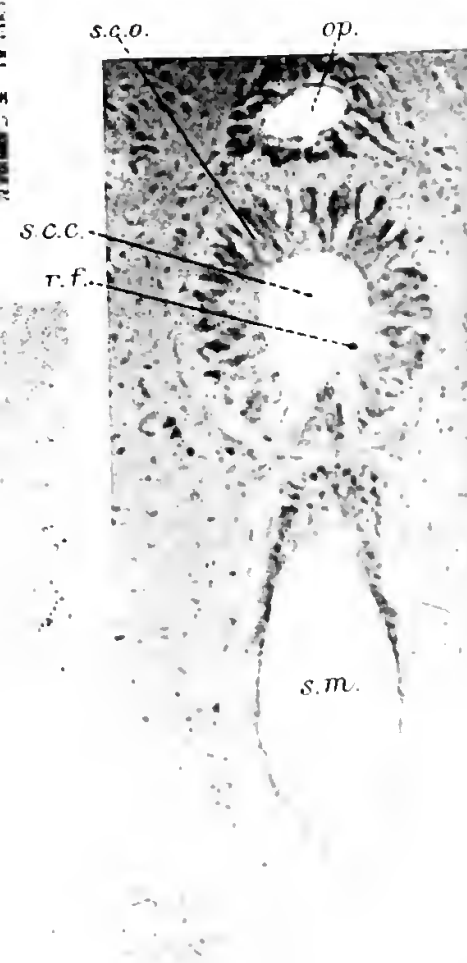
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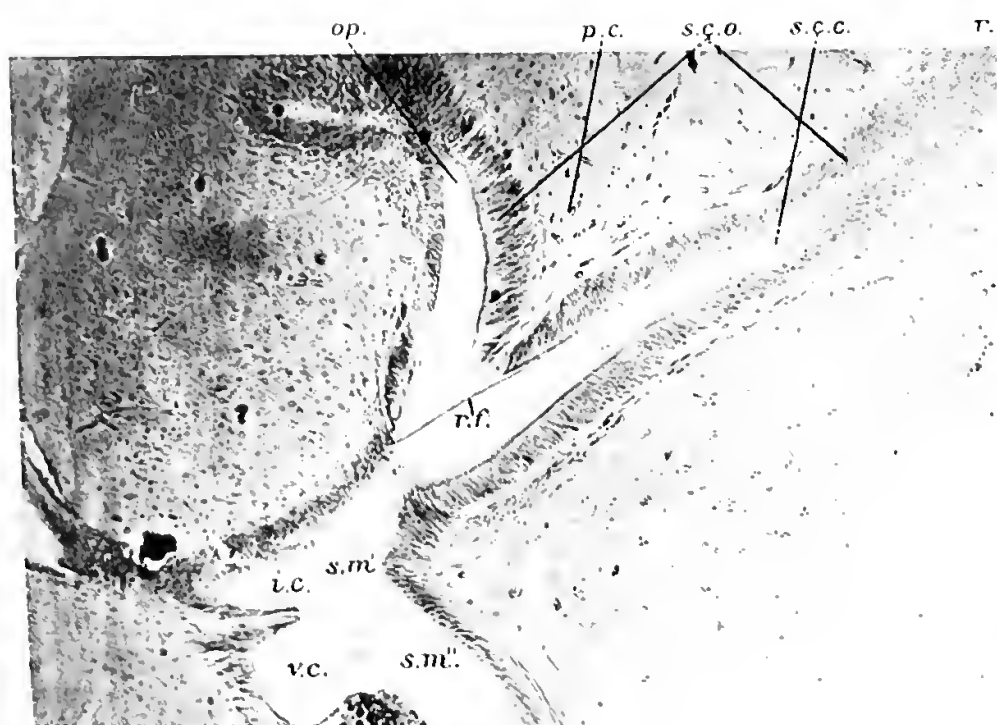
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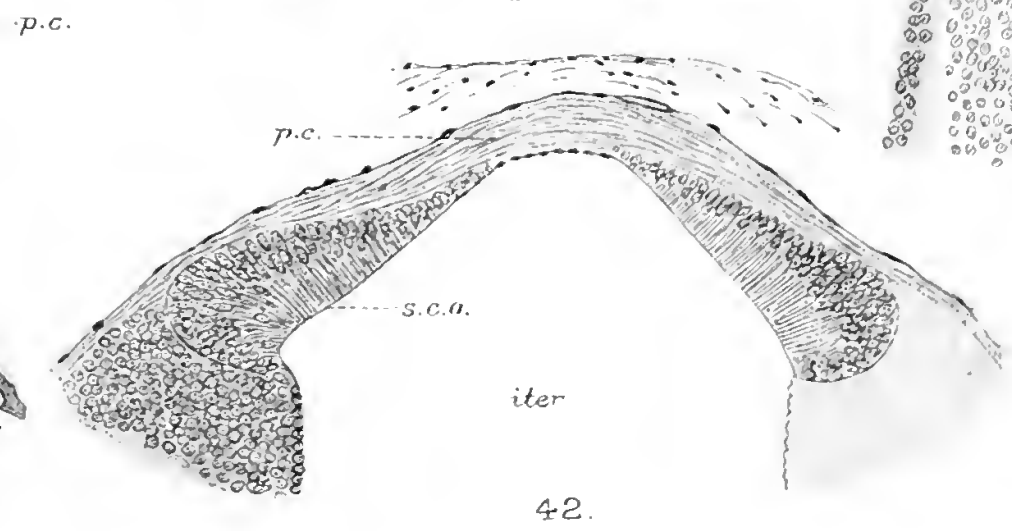
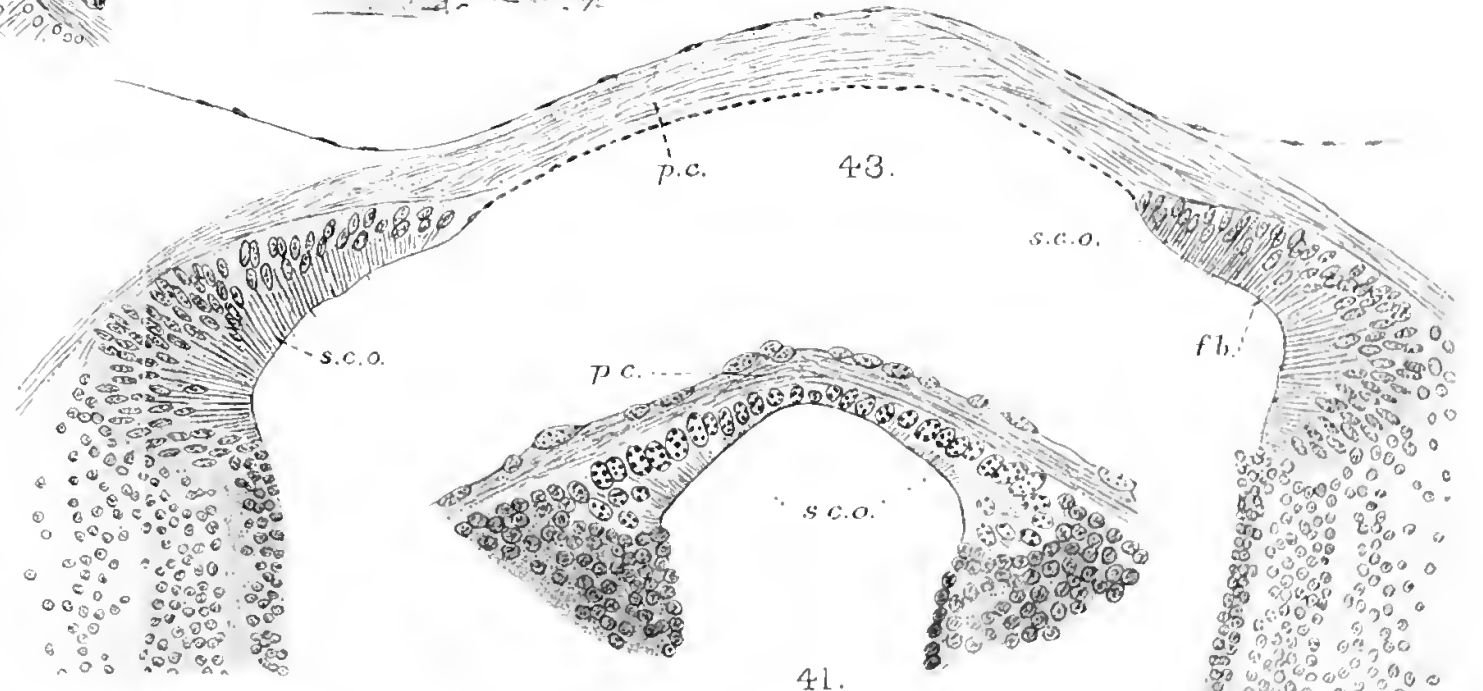
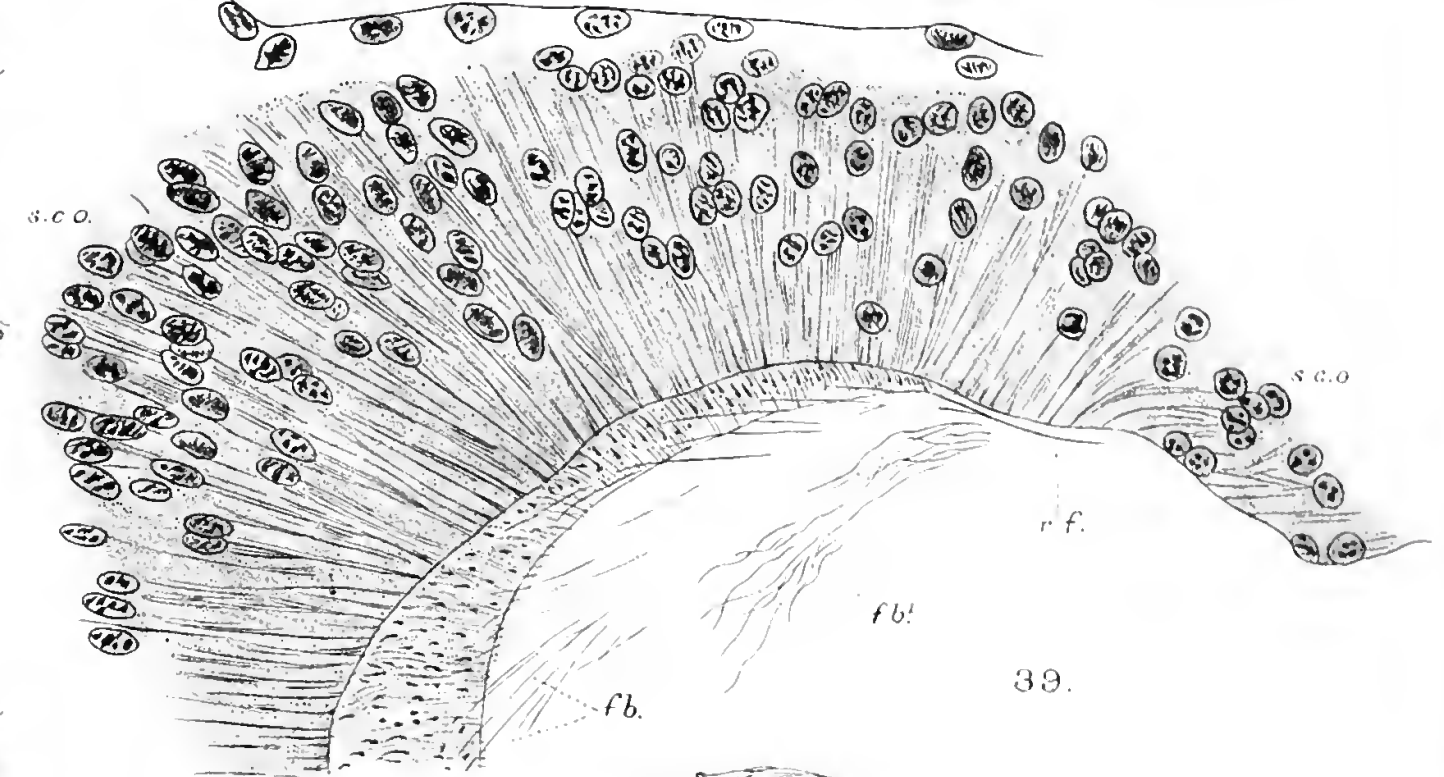
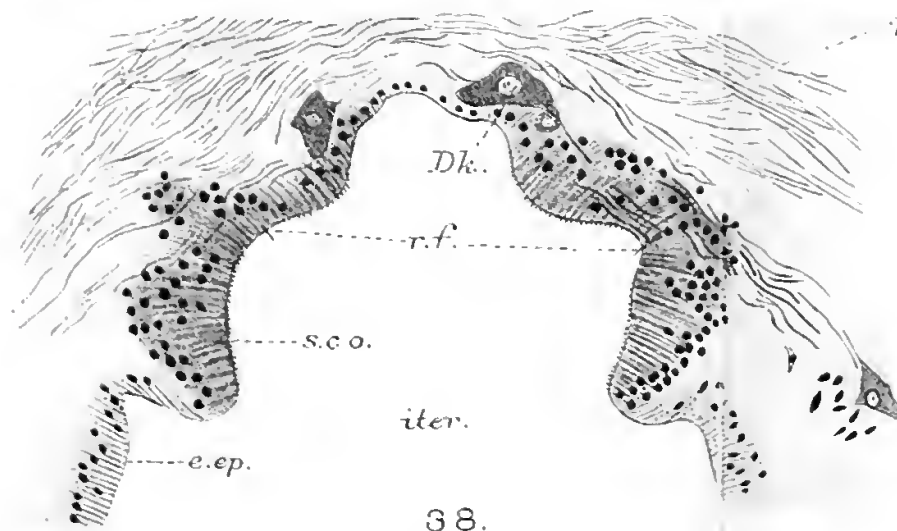
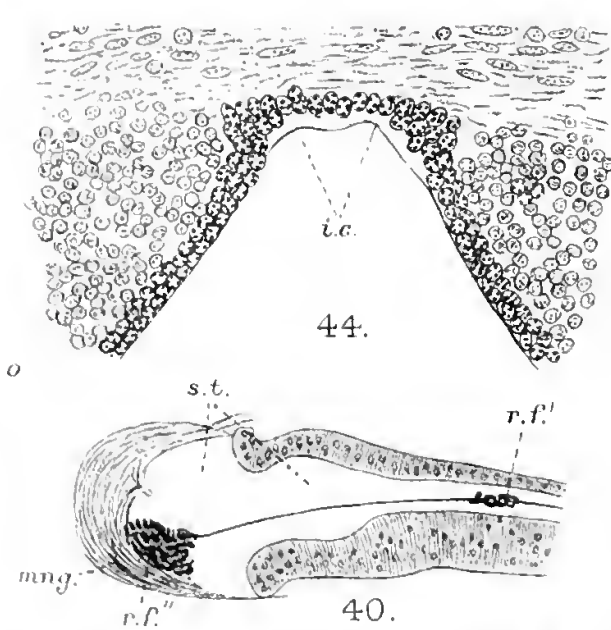
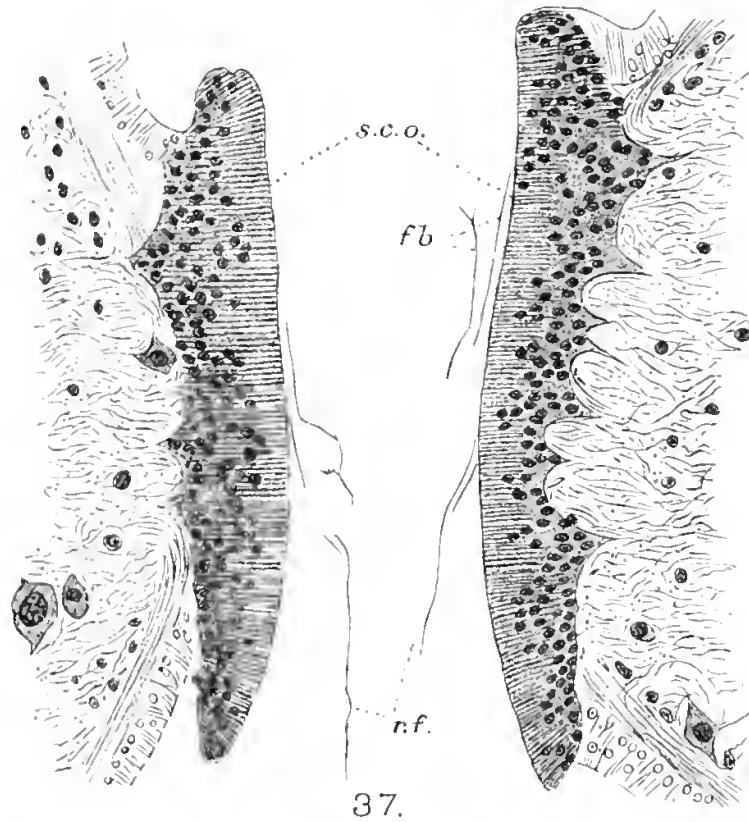
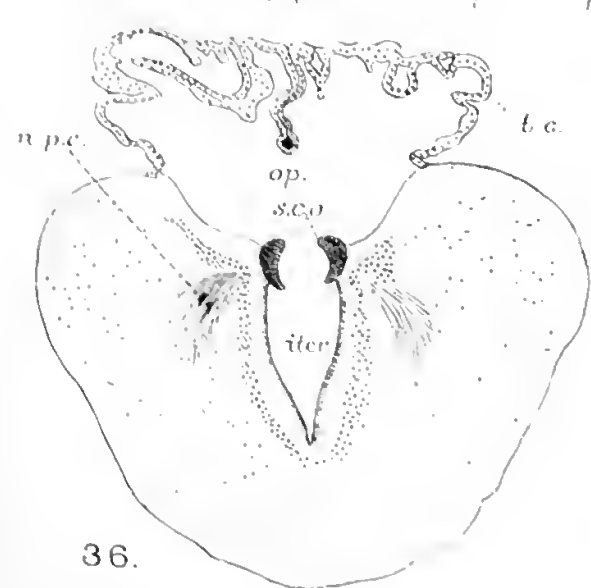
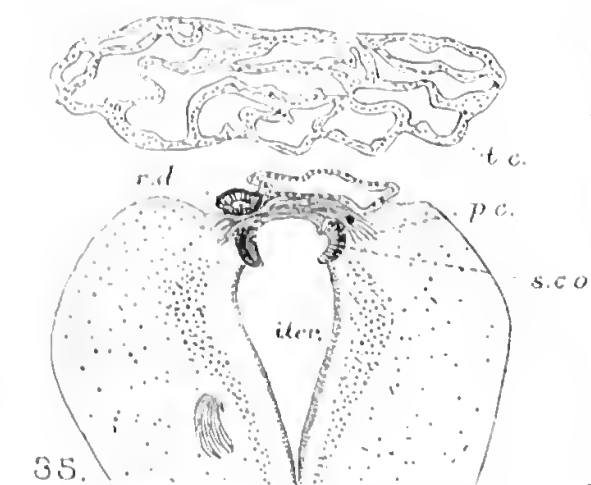
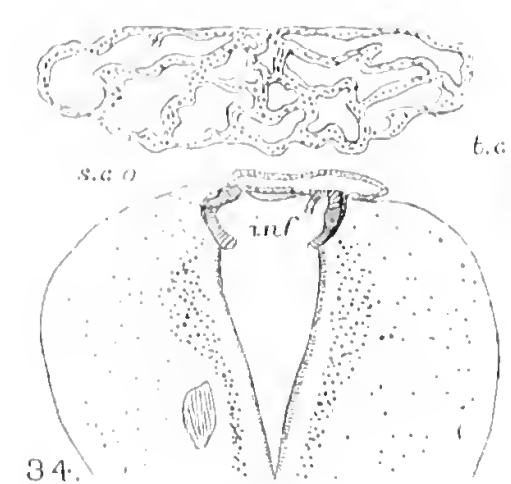
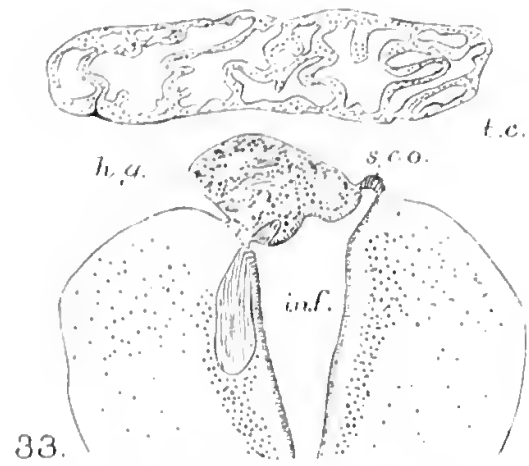
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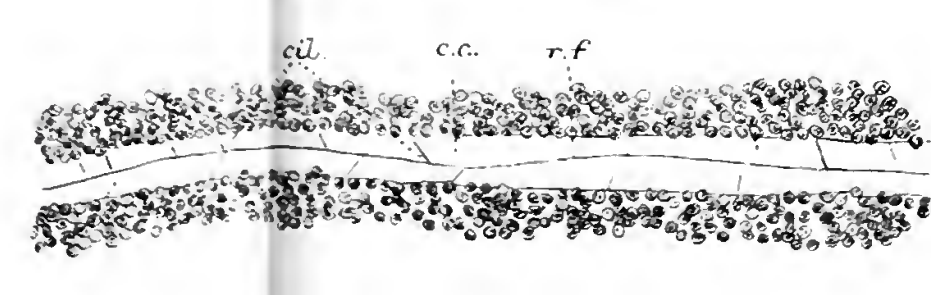
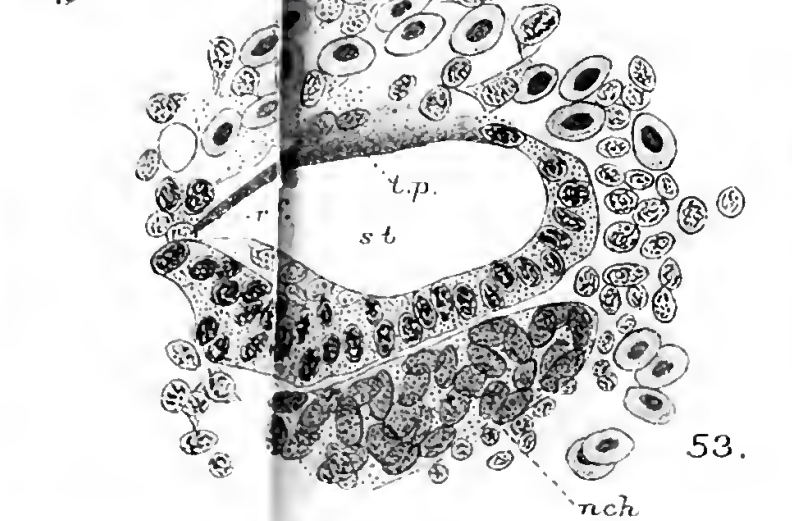
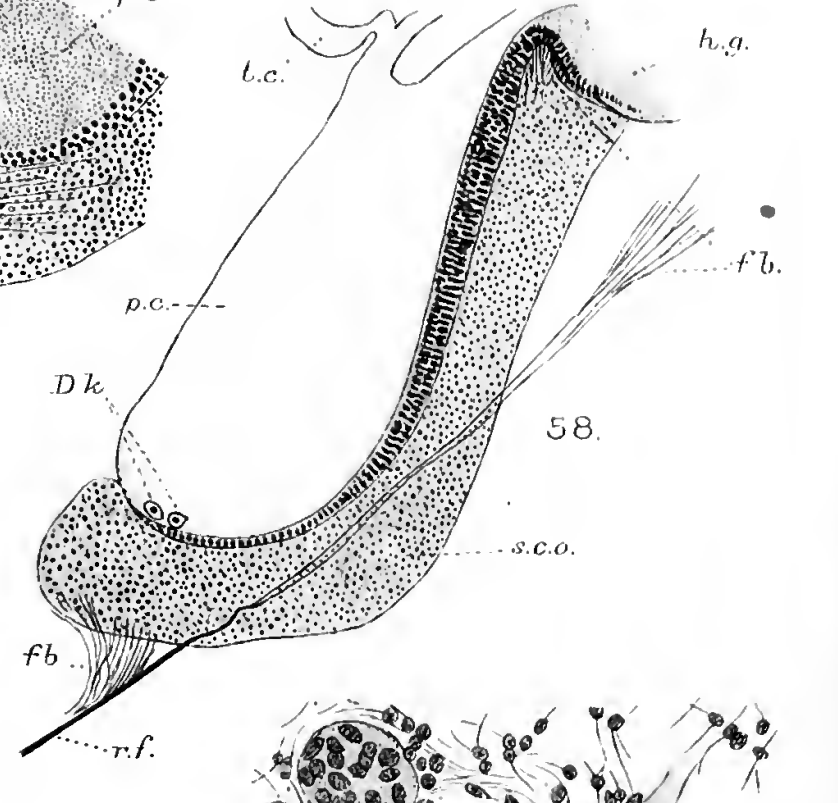
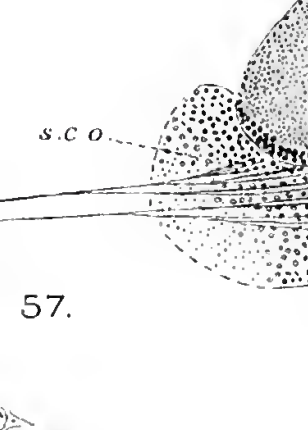
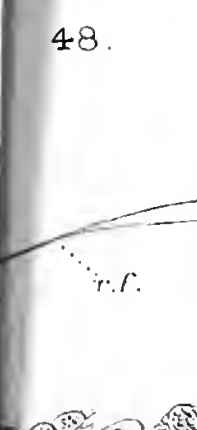
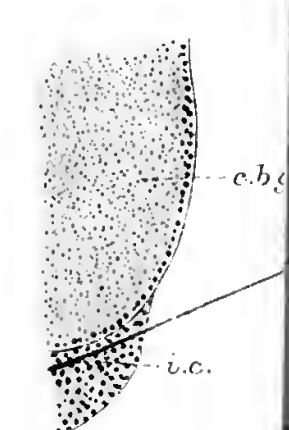
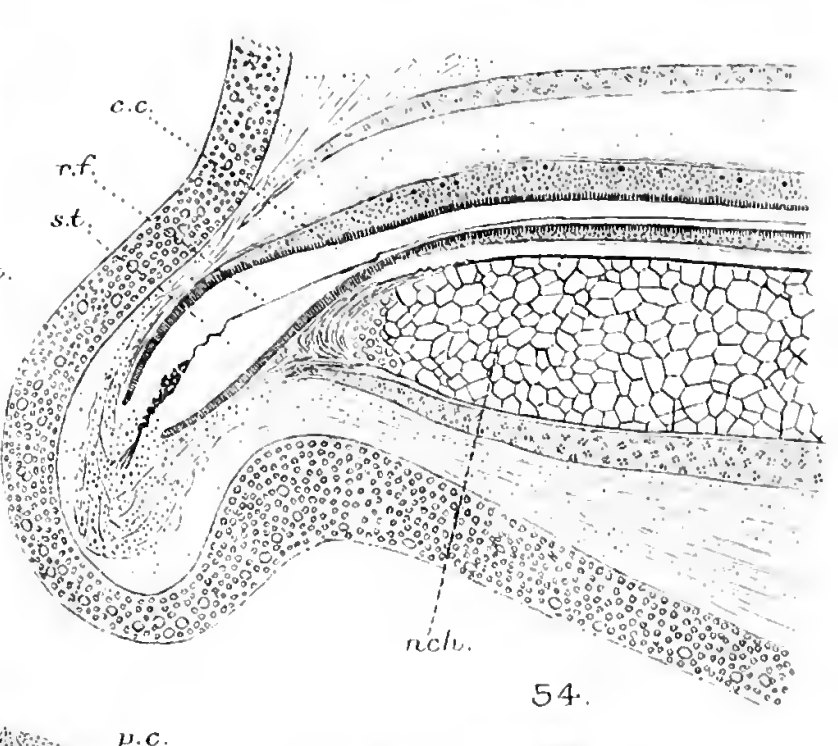
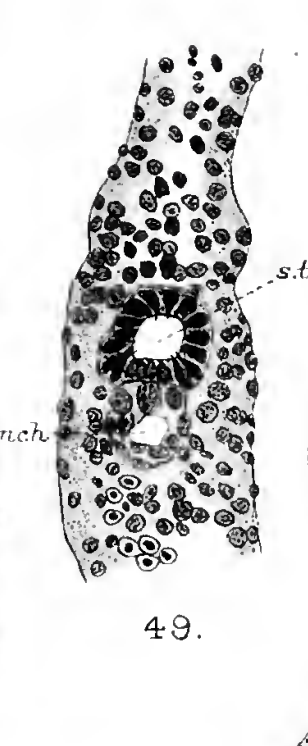
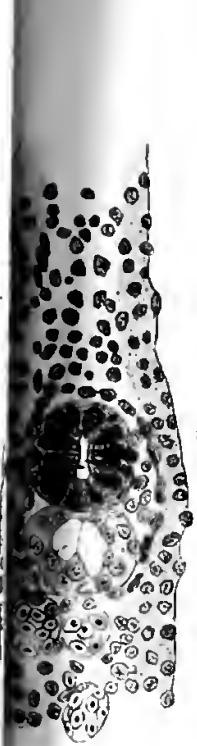
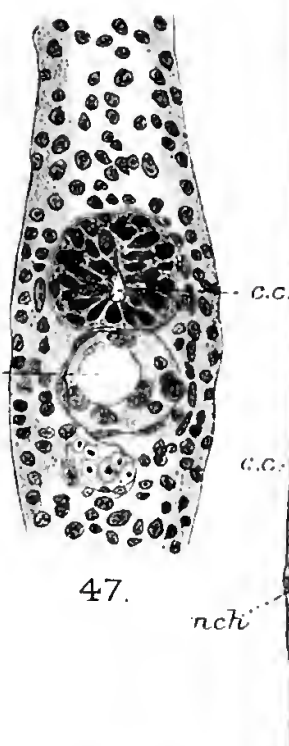
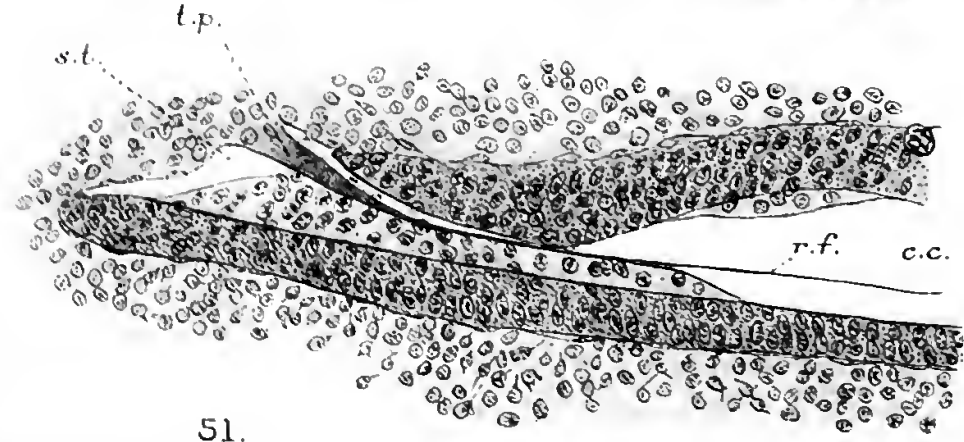
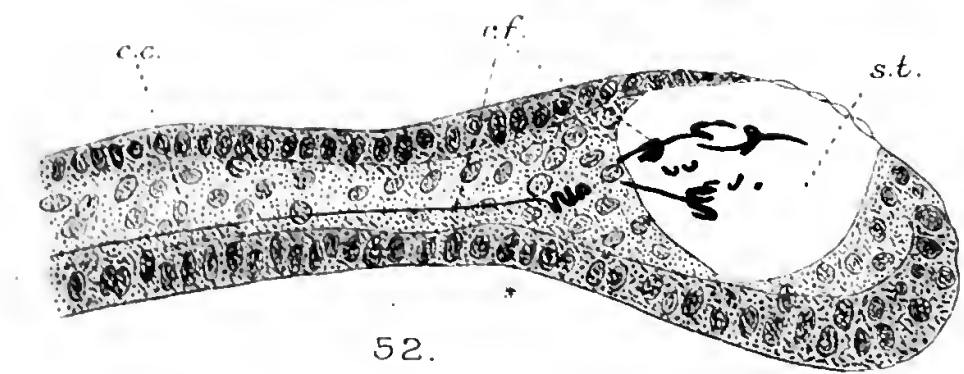
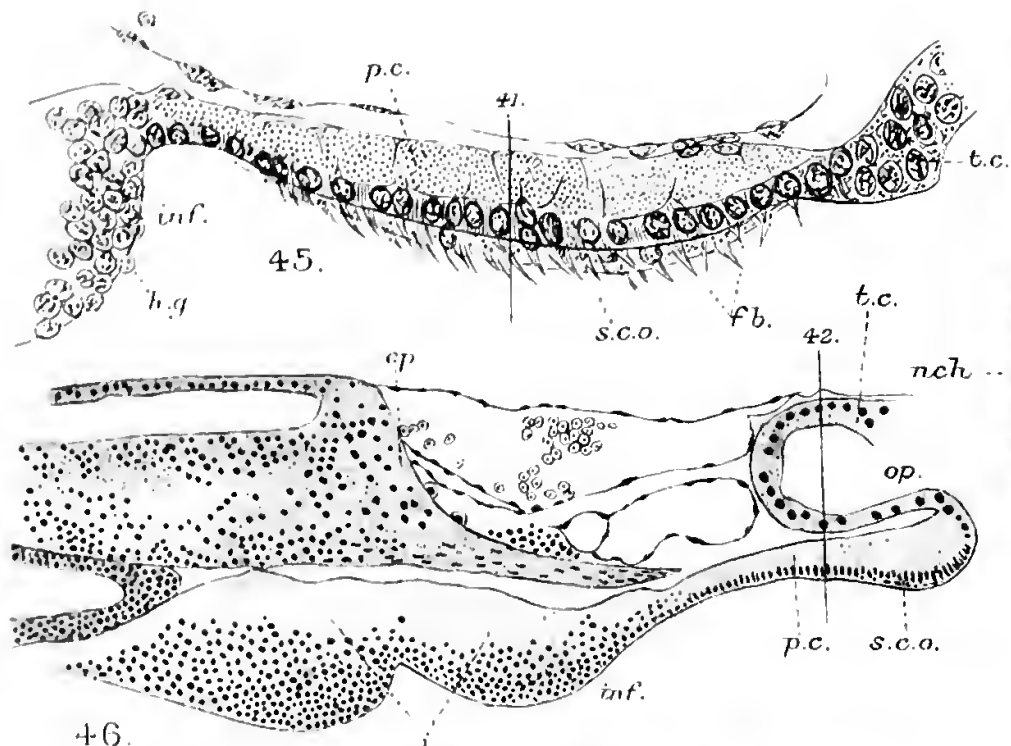




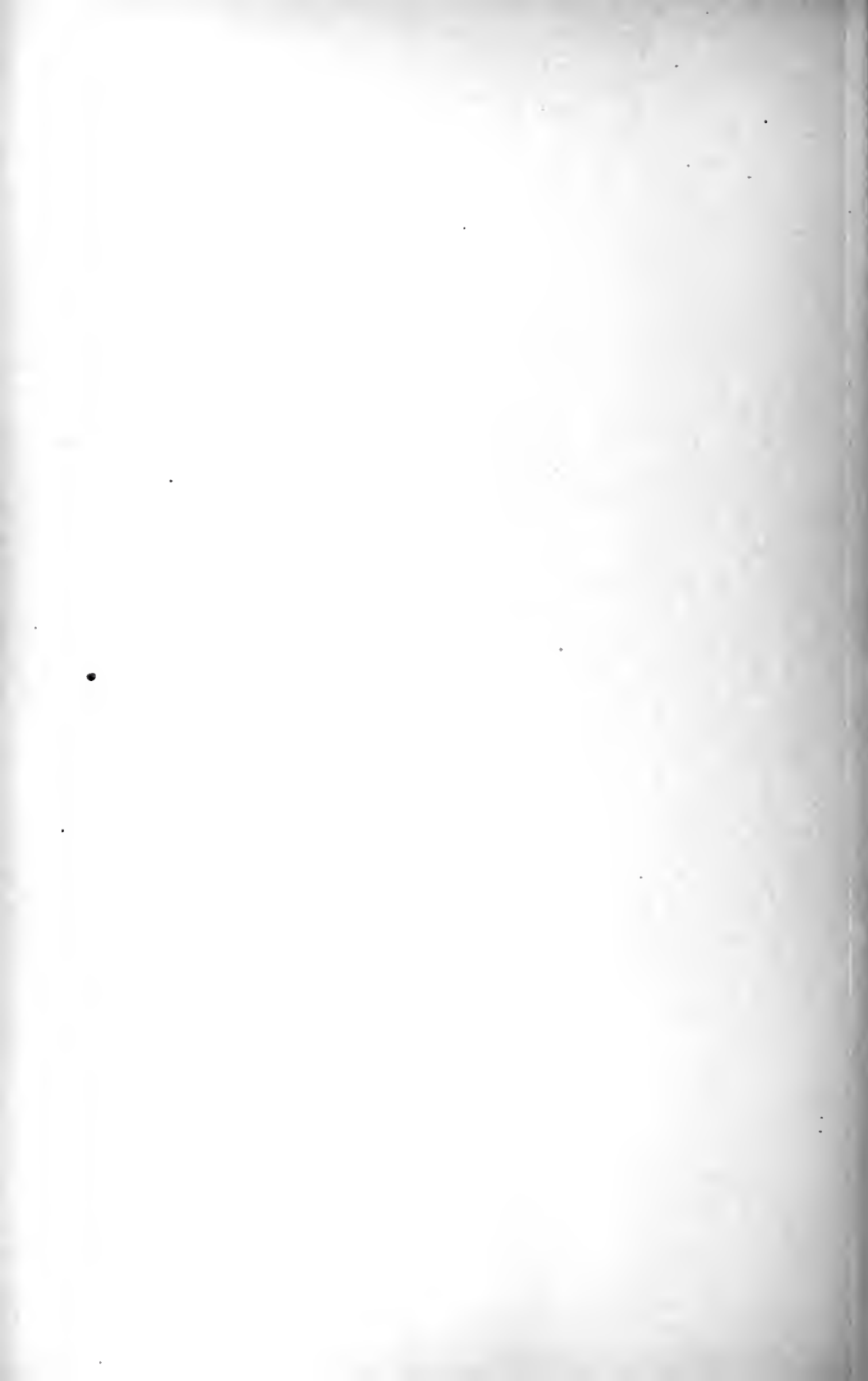












## Loxosoma loxalina and Loxosoma saltans —Two New Species.

By

**Richard Assheton, M.A.,**

Lecturer on Biology in Guy's Hospital, University of London.

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With Plates 6 and 7 and 4 Text-figs.

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THE genus *Loxosoma* is remarkable among Polyzoa in having the lophophore placed more or less obliquely to the main axis of the stalk instead of being at right angles as in other Polyzoa, and in being solitary, for the buds, which are formed readily enough, drop off before they have reached any great size, and attach themselves to the surface of some other organism or neighbouring object by means of diverse forms of adherent arrangements in the end of the stalk or foot, which varies a good deal in shape. The species, of which rather more than a dozen have been described, are all commensals living fixed on to other organisms or upon the tubes inhabited by other organisms, which may be Polychætes, Sponges, Ascidians, Gephyreans, and probably other animals as well.

Among these there is a form called *Loxosoma annelidicola*, which was originally mistaken for a Platyhelminth, and called *Cyclatella annelidicola*, having been found by P. J. Van Beneden and C. E. Hesse in 1865 on certain Maldanid Polychætes, but which subsequently was recognised as an Entoproct, and so described by Prouho in 1891.

Prouho found the species on the Clymenians *Nicomache lumbricalis* and *Petaloproctus terricola*.

More recently a species of *Loxosoma*, the first actually described from the American side of the Atlantic, has been made the subject of an interesting paper by N. S. Nickerson, and named *L. Davenporti*, also from the tube of a Maldanid, namely *Clymene producta*. This species clearly has close affinities with the other Maldanid *Loxosoma*, *L. annelidicola*, as is shown for instance, by the possession of the wing-like expansion of the body and the arrangement of the foot muscles, but is quite distinct in many characters, such as the far longer stalk, peculiar epidermic "flask organs," the more numerous tentacles, and the curious "mammary organ" which the female of *L. Davenporti* possesses. The two new species which form the subject of this present paper are also from the tubes of Maldanid worms, and, as one might expect, bear certain resemblances to *L. Davenporti* and *L. annelidicola*, but they are clearly distinguishable from these, as may be noted from a glance at prepared specimens.

#### LOXOSOMA LOXALINA.

*Loxosoma loxaliua* was found in September, 1909, in the Sound of Mull, near the entrance to Loch Aliue, in association with a Maldanid, of which I have only an imperfect specimen, and which I have been unable to identify.

**External Characters.**—It is characterised by its long stalk as compared with the other known Maldanid associates, the stalk being longer than the body and calyx together, though very much shorter than the stalk of *L. Phascolosomatum*, and the presence of curious projecting glands placed with some regularity on the sides of the body (fig. 1), which, though more numerous and smaller, are no doubt comparable to the "flask organs" described by Nickerson on *L. Davenporti*, although they occupy a different position. Ectodermic glands, unicellular or multicellular, occur on other species, e.g. *L. Tethyæ*, *L. crassicauda*, and *L. phascolosomatum*, though in these cases they are sunk

entirely beneath the surface rather than raised above it, but probably all such organs may be said to be generally homologous. In *L. loxalina* they have undergone a special development and differentiation, and though projecting from the surface, do not stand out so clearly as in *L. Davenporti* or in *L. saltans*, the second species to be described here. In all specimens that I have examined these organs are generally similar; they occupy more or less corresponding positions, and are differentiated in like manner. There are usually two pairs on the calyx and two pairs on the body, and they tend to give the animal a somewhat angular appearance. Sometimes extra pairs occur on the body. There are none on the stalk, which is sharply marked off from the body. In *L. Davenporti* they are borne about the spot where the body passes into the stalk. Although glandular is a convenient term to use, there is no sign of any secretion exuding from them. Figs. 1, 15, 16 and 17 indicate clearly enough the structure of these organs, but their function must remain for the time being a mystery. Fig. 1 represents the most usual condition. I have never found less than the four pairs here indicated, but I have in some specimens seen additional pairs. The two pairs on the edge of the calyx are less prominent than the others, and are not very different from the unicellular glands of Salensky, *L. tethyæ*. The lower pairs are either connected with, or lie in close juxtaposition to, rows of large deeply stained cells (figs. 1, 3, and 6 [*k.*]). On the body there are two pairs, the upper of which is on the ventro-lateral surface at the level of the lower margin of the two appendages of the stomach. The lowest pair of all is different from the rest, and is never absent. Whereas the others vary a good deal in form and degree of development, this lowest pair is constant. Each organ consists of a group of long cells, the nuclear ends of which are deeply imbedded in the body, and the outer ends, projecting a short distance beyond the surface, form a conical eminence with a small crater-like depression on the centre. Fig. 17 (*a.*) represents, in diagrammatic form, the structure of these and the other



epidermic glands of *L. loxalina*, and may be compared with the figures of sections of the similar organs of *L. saltans* (figs. 15 and 16), which resemble far more closely the "flask organs" of Nickerson on *L. Davenporti*. It is pretty clear that they are all essentially similar morphologically, whatever their function may be. From the distinct character and constancy in position of the lowest pair we may assume that these particular ones have a special function. They are more deeply set than the others.

The foot is circular and devoid of any special gland such as *L. tethyæ* and *L. leptoclini* possess. It has, however, a peripheral row of unicellular structures, which may be mucous or some form of adhesive gland which project beyond the general contour as the toes of a frog project beyond the web (fig. 13). But quite possibly they may be of firmer consistency and serve as stiffening rods.

The lophophore of this species as well as *L. saltans* is not circular in outline, but slightly indented along the oral region, at first sight suggesting the condition in the *Phylactolamata*, but in them the inflection is of the anal region. The tentacles in every case I have counted number sixteen, and are not all of the same size. Four along the anterior or indented part are longer than the rest, and a gap occurs between the two inner ones of the four. The same characters are seen in *L. saltans*, and apparently also in *L. tethyæ* according to Salensky, though only two tentacles are shown by his figures to be longer than the others.

Alimentary Canal.—The whole body is very much compressed in the oro-anal axis, but does not show any features very strikingly different from those of other species of *Loxosoma*. The rectum is carried far along the hood of the lophophore, so that the anus lies near the rim of the lophophore as in *L. crassicauda*, but unlike *L. Davenporti*, where the rectum ends about the centre of the lophophore hood. The rectum is wider than the intestine and its walls contain cells with brown inclusions, which are probably excretory products (vide *L. saltans*). The whole body being longer in *L. loxa-*

lina than is usual, the alimentary canal is longer, and the lower or proximal part of the stomach is more distinct and forms a triangular or conical-shaped chamber. There are well-marked lateral swellings which form the glandular portion of the alimentary system (fig. 1, *ld. pd.*).

The mouth is bounded posteriorly by an epistome and anteriorly by the edge of the lophophore, which in the middle line is raised up into a little knob which in *L. saltans* bears stiff hairs. The mouth leads into a wide funnel-shaped chamber quickly narrowing into an œsophagus lined by long cilia. The cells which form the walls of the œsophagus contain a dark olive-green pigment lying deep in the cells and which occurs nowhere else in the animal.

There are really two pairs of diverticula from the alimentary canal, an anterior or proximal pair, though actually lying nearer the foot than the lophophore region, and strictly lateral, and a posterior or distal pair less sharply constricted and lying rather more towards the ventral surface. Fig. 1 of *L. loxalina* and figs. 10, 20 of *L. saltans* illustrate this well enough.

The alimentary tract is ciliated over a great part of its surface, though in certain regions the cilia are longer than in others. The whole of the œsophagus bears long cilia, the lower chamber of the stomach is lined with short cilia, while the diverticula are devoid of them. The intestine is ciliated throughout by short cilia. The character of the cells forming the walls of the alimentary canal seem to be similar in the two species, *L. loxalina* and *L. saltans*, and some further details are given in the next section.

I shall not dwell upon the histological details of *L. loxalina*, as although I have over a hundred specimens of this species, they were all obtained from the tube of a single worm, and were preserved alike in Perenyi fluid, which is not a suitable reagent for fixing this animal's tissues. In fact the specimens were not discovered until some time after preservation in the bottle containing the sand tube and portions of the Maldanid worm. I have not succeeded in coming

across the species again, so that I have not seen it alive. The Maldanid worm was the only one obtained, and that was got some feet below low-water level of a spring tide.

**Nervous System.**—The only part of a nervous system observed is the paired ganglionic mass which lies between the intestine and the reproductive gland as shown in fig. 3, in a position corresponding exactly with that of *L. saltans*, fig. 14, and other species.

**Excretory System.**—The poor histological condition of the specimen makes it very difficult to determine the character of the excretory organs. There are certain structures which are probably of that nature which lie in the body just above the glandular expansion of the stomach. Firstly, there is a pair of large rounded masses of deeply staining cells which have rather the appearance of yolk-glands or testis, and are no doubt part of the reproductive system (fig. 6). Closely applied to these and extending from them to the skin are very peculiar cells arranged in tiers, pagoda-like, as in fig. 1 (*k.*), and fig. 6 (*k.*), the larger cells being at the bases. Next the skin are some small rounded cells. It is not possible to make out a duct, nor is it easy to make these structures correspond with anything hitherto described as excretory organs of polyzoa. Nevertheless, as will appear in the next section, it seems to me possible to derive them for the structure which must be regarded as excretory organs in *L. saltans*.

**Reproductive system.**—This consists of a pair of large gonads (figs. 1 [*g.*] and 4 [*g.*]), from which wide ducts run inwards towards a mass of large granular cells lying in the median plane just behind the nerve ganglion and œsophagus. This mass may be of the nature of a shell-gland (c f. Nickerson), or possibly a yolk-gland, and from it a single median duct runs to open into the atrium between the epistome and the lophophore.

The gonads are not very well preserved, but at any rate I can say that each is a more or less spherical sac with thick walls and a small central cavity from which the gonoduct runs. The walls bear the reproductive cells, and I am inclined to

think that they are hermaphrodite; but I do not wish to commit myself absolutely. Figs. 3, 4 and 6 illustrate these points. There seems to be an anterior duct or opening (fig. 5, *k.d.*) on the epistome. Whether this is renal or reproductive I cannot determine.

**Muscular System.**—The contractile tissue is well developed in connection with the stalk, foot and tentacles. The long peduncle contains longitudinal fibres which run parallel with the surface between the body and the foot, and closely applied to the surface. At the proximal end some of these fibres bend across and become continuous with the walls of the alimentary canal. I cannot make out that they divaricate in so marked a manner at the distal end in reaching the foot as one would expect from the condition described in *L. annelidicola* or *L. Davenporti*. There is a strongly developed circular band of fibres developed on the inner rim of the lophophore.

The surface of the stalk contains longitudinal rows of larger cells, and one especially well-developed row extends down the mid-dorsal line. This also occurs in *L. Davenporti* and others.

#### LOXOSOMA SALTANS N.S.

An allied form of *Loxosoma* living also in the tube of a Maldanid occurs farther north in the sands of the shores of Skye, in the neighbourhood of the Kyle of Loch Alsh, some three or four feet below low-water level.

This is sufficiently different from *Loxosoma loxalina* of the Sound of Mull to deserve a different specific name, though it, together with *L. Davenporti* and *L. annelidicola* and even *L. crassicauda*, form a group showing special affinities. All these species live commensally with tubicolous Polychætes. The difference is distinctly indicated in the two figures, 1 and 20 on Pls. 6 and 7, which are drawings of typical specimens of the two forms after preservation. Fig. 10 gives a better idea of the living animal, *L. saltans*. The Skye

form is shorter and broader, and has a smaller body in proportion to the size of the lophophore than *L. loxalina*. The curious gland-like processes are quite different. They are less numerous and far larger in the Skye species, in which I have never found more than two present. They arise from the ventro-lateral surface about half way down the body—whereas in *L. loxalina* there are usually four pairs set more on the lateral edges. They resemble very closely the flask organs of *L. Davenporti*, but are larger and placed higher up on the body.

In the Skye species these curious organs may be altogether wanting, or one alone may be present. They are pedunculate, whereas in *L. loxalina* they are partially sunk beneath the surface. Specimens with buds, which buds arise from the ventro-lateral surface anterior to the spikes, are found with or without spikes, so that there is no correlation between these organs and the buds. Figures 15 and 16 represent sections taken rather obliquely through these organs. It will be seen how in fig. 15 the epiblast is ruptured at one side, thus indicating how easily they may become detached. They are in all cases entirely epidermic.

The character which suggests the name I propose for the species *Loxosoma saltans* is the peculiar mode and highly developed power it possesses of locomotion. When freshly taken it is extremely active, moving over the body of the worm or along the lining of the tube in a manner fascinating and unique by a series of gymnastic efforts, which combine the agility of the kangaroo and the deliberation of a geometer caterpillar.

It is possibly in correlation with this habit that there is a modification of the lophophore, which is not perfectly circular as is usual among the Entoprocta (except *L. loxalina*), but is elongated in the oro-anal plane, and shows a slight tendency to a separation into right and left halves, and is inflected along its oral region (fig. 19).

The tentacles, as a rule, number sixteen, so that we have a right and left series of eight very slightly separated from

each other by the occurrence of a rather wider interval between the first of the right and the first of the left and last of the right and last of the left. A papilla bearing stiff hairs lies between the two series at the oral end. The special modification in connection with the habit of jumping is in the four oral tentacles, that is to say the first and second of the two series, forming the four on the inflected edge of the lophophore. These are longer than the rest, and when closed down over the mouth fold outside and across the tentacles of the lateral parts of the lophophore as in fig. 19. These four usually work together and are raised off the floor of the calyx quite independently of the others. When the animal moves its position these four tentacles play a part in the action.

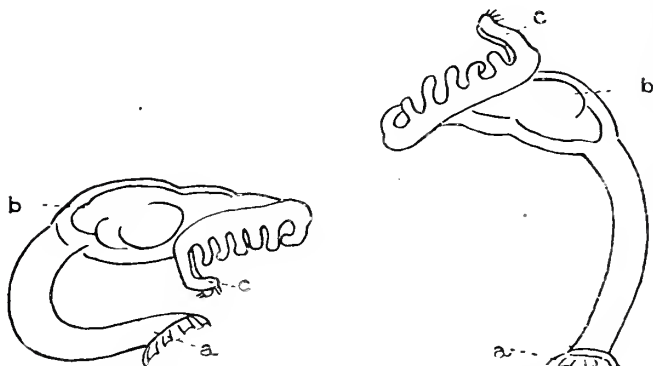
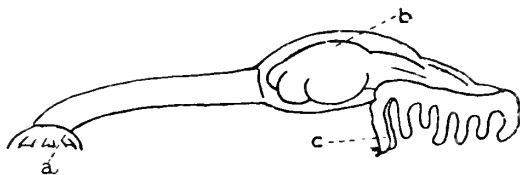
The stalk of the animal is extremely strong and muscular, and can swing the body about in any direction with ease and rapidity. The calyx may be directed outwards from its point of attachment, so that when expanded it shows a campanulate form as in fig. 10, which resembles the ordinary *Pedicellina* condition rather than that typical of the genus *Loxosoma*, and in this respect it is more like *Urnatella*.

When lying prone, as presumably it must often do while between the worm and tube, we may suppose that it lies with its oral surface towards the object to which it is attached, as this is the position it more usually takes when not waving outwards. Prouho's figures seem to show that *L. annelidicola* lies the other way. It is in this position that locomotion is possible. This is effected by the animal bending down its body and lophophore into contact with the surface over which it is about to move, but contact is made only by the tips of the four large oral tentacles, which are provided with special stiff bristles on their outer tips (fig. 10), which do not occur in the other tentacles. These no doubt serve to give it a hold. The four tentacles are then suddenly bent backwards, that is to say outwards, and at the same moment the peduncle is whisked forwards with great rapidity, and the adhesive foot applied in a fresh situation, as in Text-fig. 1.



It appears to be a distinct jump; there is a moment when neither foot nor tentacles are in contact with the surface over which the animal is progressing. It is not quite like the looping of a caterpillar; it is more like the action of the boy playing "leap-frog." The animal can either bring its foot directly forward, or swing it horizontally according to space.

TEXT-FIG. 1.



TEXT-FIG. 2.

TEXT-FIG. 3.

Three successive attitudes of *L. saltans* during the act of jumping. a. Foot. b. Gut. c. Tentacles used in jumping.

As I have not succeeded in obtaining *Loxosoma loxalina* alive, I cannot say whether it leaps, but from the fact that there is the same difference between the oral tentacles and the others it seems probable that it does. Salensky's figure suggests that a similar modification also occurs in *L. tethyæ*.

As far as I know no such agility on the part of a Polyzoon has been observed before; *Cristatella*, of course, is known to move, but that is motion of a very different kind. It seems

possible that a somewhat different though less lively mode of progression may be possessed by *L. Davenporti* from what Nickerson says, though there is no modification, nor similar action, of the four oral tentacles. Nor is the oral part of the lophophore rim indented. In *L. Davenporti* there are certain cells at the base of each tentacle on its outer surface, "the cuticula over the cell" being "thickened to form a flattened or sucker-like protuberance." Nickerson continues (p. 355): "If a number of specimens in clear sea-water in a smooth glass vessel be observed attentively, individuals may often be seen lying on the dorsal surface with the lophophore fully expanded. If a current of water from a pipette be directed against an animal in this position it becomes evident at once that the creature is attached quite firmly by the lophophore margin; and though the foot end may be lifted up by the motion of the water, the hold of the animal is loosened only by a very strong current. This observation makes it evident that the cells in question serve as a means of attachment. They are to be regarded as unicellular suckers, which are of use to the animal in enabling it to keep a hold upon its host while changing its foot attachment."

**External Form.**—The living animal is almost transparent and colourless except for the excretory organs and alimentary canal, which is throughout slightly yellow, and at certain points it is strongly pigmented and shows up brightly. The pigmented portions are the two distal and lateral swellings on the stomach, which vary from a bright yellow or orange to brown (fig. 19), and the latter part of the alimentary canal or rectum which is wider than the intestine. The two smaller and proximal diverticula of the alimentary canal, which in sections are seen to take stain more readily than any other part of the alimentary canal, are almost quite devoid of any colour in the living animal. Fig. 19 illustrates the colour of the living animal and fig. 10 the general form and character. In this specimen there were two large pedunculate "flask organs," of which one is shown in fig. 10,

and two buds; one of these is well advanced. The figure shows the way in which the lophophore expands and the small tentacles spread out, though always slightly curved inwards. The four oral tentacles are usually moved together and often thrown back as in the act of jumping. None of my specimens, nine in number, had more than two buds.

The stalk is shorter than in *L. loxalina*, and the foot is slightly larger, but resembles the foot of *L. loxalina* with its toe-like organs very closely. I think there are twelve to sixteen of these organs.

**Alimentary Canal.**—The general character of the alimentary canal is indicated by the figs. 11 and 12. It differs from that of *L. loxalina* only in the greater size of the distal, more ventral diverticula, which are the most pigmented parts, the long cells being filled with yellow granules. Fig. 11 is a slightly oblique section which passes through the œsophagus and the edge of one of these thick-walled diverticula, which are composed of elongated cells with the nucleus at the outer end and loaded with spherical yellow inclusions (figs. 11 and 22). I think the gut lining is throughout a single cell in thickness which cells vary greatly in length. The œsophagus opens into the stomach low down on the anterior face. As in *L. loxalina*, the anterior and posterior walls contain granules of dark colour, but they are of a deeper brown and less refractive than in the former species. The intestine leads from the stomach by a gradual narrowing of that organ, but along the whole dorsal wall of the stomach there is a groove (which is continuous with the lumen of the intestine) of low ciliated epithelium. This in its turn is continuous with the conical lower apex of the stomach and with the entrance of the œsophagus, all of which is ciliated. The only parts not ciliated are the four glandular tracts. The longest cilia are in the œsophagus and at the entrance of the œsophagus to the stomach. Figs. 11 and 12 illustrate the character of the epithelium of the apex of the stomach, where it is low, columnar (and I think really ciliated) with a few mucous glands (*mu.*). The section also shows the

proximal diverticula of long cells with small nuclei at their bases and filled with fine granules of quite a different character to the cells of the larger distal pair. The cells of the apical part of the stomach and the intestine contain many granules at their basal ends which are blackened by osmic acid and are probably fat-globules. This part of the stomach may be regarded as absorptive (figs. 11 and 12). The characters of the proximal pair of diverticula suggest secretion of some digestive juice, the cells being filled with very fine granules not unlike the granules of vertebrate pancreatic or salivary gland-cells. The margin of these cells or of some of them are irregular like those of mucous cells or other actively secreting cells, and probably indicate the pouring out of secretions into the general digestive cavity. These surfaces are devoid of cilia.

On the other hand, the larger, thicker-walled diverticula on the more ventral surface, which are yellow in the living animal, are formed of long large cells loaded with spherical masses of various kinds, some staining purple with hæmatoxylin, others yellow and various shades of brown. Some stain intensely with thionin, others not at all. The characters indicate active constructive metabolism, and suggest a function to these parts of the alimentary tract more comparable to that of a liver.

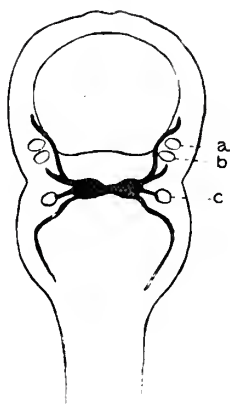
The intestine has a narrow lumen, is circular in transverse section, and has a thick, homogeneous wall richly ciliated until it reaches the hood of the lophophore. Here the lumen widens, and fæces are collected, and the walls contain yellow pigments probably of an excretory nature. The anus is extremely small. I presume that it exists, but in no section have I been able to see it as an actual opening. Its position is indicated, however, by a proctodæal depression and a suture which is probably caused by its coalesced edges.

Nervous System.—The central nervous system, as in *L. loxalina*, is a conspicuous object lying between the intestine and reproductive gland (fig. 14, *n.g.*). It consists of a pair of ganglia connected by a thick band of nerve-fibres. Peri-

pheral nerves radiate from the ganglia to the lophophore and body-kidneys (if such they be), the body-wall, gut-wall, and lophophore, which in plan can be represented by the accompanying diagram. But I must confess that I have not succeeded in tracing out the termination of the finer branches.

In specimens which have been left alive in sea-water containing methylene-blue there are rows of cells along the stalk which stain blue (fig. 21), and one particularly prominent row runs along the mid-dorsal line of the stalk (fig.

TEXT-FIG. 4.



*L. saltans*. Nervous system (thick lines). *a, b*. Lophophoral kidneys. *c*. Body kidney.

21). This prominent row is confined in *L. saltans* to the stalk; it dies away as the stalk passes into the body and also as it joins the foot. A similar row of cells has been described by Salensky for *L. crassicauda* and *L. tethyæ* (though Harmer (4) did not find it in *L. tethyæ*), and by Nickerson in *L. Davenporti*. Salensky considered them to be gland-cells, and Nickerson appears to agree with him, although "no conditions have been observed which point to a discharge of the contents." It seems to me not improbable that these cells may be nervous, or neuro-muscular, forming perhaps a kind of muscle plate centre of nervous impulse in

connection with the highly developed and rather complex character of the movements of which the stalk of *L. saltans* at any rate is capable.

The paucity of specimens prevented me from making an exhaustive study of the nervous system of this interesting little acrobat.

I cannot see in these cells any real resemblance to gland-cells. Their arrangement as a regular single file is more suggestive of co-ordinate action than glandular activity, which more usually is connected with diffuseness and irregularity of form.

One speaks of the foot as being an adhesive disc, and, as Nickerson says, containing unicellular gland-cells. I cannot believe that the toe-like cells which project round the edges of the foot are adhesive in the sense that they excrete any adhesive material. The rapidity and ease with which the animal relaxes its hold and swings its foot round and takes a new grip suggests a complicated nervous and muscular action, involving some action, such as suction, as the means of attachment rather than a simple adhesion by secretion. The so-called glands (fig. 13) may be of the nature of rods which stiffen the expanded rim of a sucking disc. One can imagine that such an arrangement would enable the animal to obtain hold and relax with great facility.

Sense-hairs are borne by the tentacles, and upon the hypostome and on the lophophore at the point of its greatest inflection in the oral region.

**Excretory Organs.**—I endeavoured to find some evidence of flame-cell tubes in the living animal, and although not working under very favourable conditions, I shall be surprised if such organs are found by anyone else in this species. I could find no trace of any ciliary action in any part of the animal excepting the alimentary canal and tentacles.

Animals which have been living some twenty-four hours in sea-water containing methylene-blue become generally coloured by the dye, but certain parts become an intense blue. When transferred back again into pure sea-water the



dye disappeared from the general tissues, but was retained by certain parts. On placing the animals into Hermann's or Flemming's solution afterwards the blue parts were intensified, and some from which the blue had disappeared stood out again in a kind of indigo blue-black.

The organs affected permanently are, I believe, the nervous and excretory tissues. The brain is coloured blue, but not very strongly; I could not trace by this method any nerve-trunks passing from it. The alimentary canal, I think, was hardly affected, with the exception of the rectum, the cells of which became an intense blue. It is hardly probable that the walls of the rectum are in any way modified with reference to the nervous system. The lines of cells on the stalk, as before mentioned, are also slightly coloured blue (which became intensified after fixation by Flemming's or Hermann's fluids). It is quite probable that the rectum cells may have special excretory functions. The other parts which are intensely coloured are a pair of organs in the base of the lophophore, and a similar pair rather lower down in the body just above the "liver diverticula."

These are probably excretory organs. I have already referred to them as the lophophore and body kidneys respectively. They, it is true, receive very distinct nerve-trunks from the brain, but their whole appearance is that of an excretory organ rather than a sense-organ. There is a pair of spaces bounded by an irregular layer of cells still lower and nearer the sides which adjoin the second pair of excretory bodies, which I have not noticed to be coloured by the methylene-blue. This latter corresponds more in position with the curious "pagoda"-like organs described as an excretory organ in *L. loxalina*.

The two pairs of organs (figs. 7, *l.k.* and 8, *b.k.*) are clearly the organs described by Prouho in *L. annelidicola* and by Nickerson in *L. Davenporti* as excretory organs. They are composed of several large highly vacuolated cells closely pressed against one another and are slightly yellow in the living animal.

I have seen no trace of any duct in connection with the body pair. In connection with the lophophore organs there is a trace of a duct (vide figs. 5 and 7, *k. d.*), but I cannot follow this tube either to the exterior, though it runs close to the surface of the epistome, nor to the cells themselves, although, as seen in the figures, it lies close against them. Pronho found a duct which was ciliated and open to the exterior, and Nickerson a similar one, but was doubtful about the ciliation. I feel pretty sure there is no ciliated duct. It is quite possible that the above-mentioned tube is a duct to the surface in connection with the lophophoral kidney, and that the much larger open space lined by irregular cells mentioned above as lying to the outside of the body-kidneys may be the duct of the body-kidney, but I cannot say whether it opens to the exterior or not.

The walls of the rectum in all probability are an important part of the excretory system. Figs. 20, 21 and 24 show how clearly certain cells of the side walls take up the methylene-blue, even more distinctly than the lophophoral and body "kidneys." In the normal condition the side walls of the rectum are yellow like the walls of the liver diverticula of the gut. In each case the colour is due to spherical bodies within the cells of varying tint, but whereas the liver-cells or inclusions are hardly affected by the methylene-blue, the rectal ones stain deeply.

In sections of the rectum one sees more clearly what the process probably is. Fig. 24 represents a section of the rectum taken transversely and stained with thionin, eosin and orange G. The cells forming the anterior and posterior walls are ciliated and do not form excretory granules like the side-wall cells, which are larger and non-ciliated. In these there are large vacuoles containing masses of granules, some of which take the thionin, others the orange stain. These grains are seen to be forming in the deeper parts of the cells (fig. 24, *ex. gr.*) and passing into the vacuoles. Some of the vacuoles (*vac.*) are deep down, others (*vac.'*) are closer to the surface, and some I have found at the surface. It is

quite likely that the vacuoles burst eventually and discharge the contained excretory granules into the rectum. I have not observed such a discharge of a vacuole in a living specimen, nor am I prepared to say that the ruptured vesicles seen in sections are not due to artificial causes, but the whole appearance of the epithelium strongly supports the view put forward above. Harmer, in his paper on the excretory processes in ectoproctous Polyzoa, describes the excretion of granules containing artificial pigment from various parts of the alimentary canal thus (p. 154): "This process takes place by the separation of small round vesicles from some part of the wall of the alimentary canal, and probably from the cæcum. These vesicles contain granules of Bismarck-brown, and may be seen in the stomach, intestine, or rectum, where they are no doubt on their way to the exterior." Then, again, in discussing, on p. 162, the nature of the natural colouring matter of the "liver" part of the alimentary canal he writes: "Without going into the question of the excretory value of the processes which take place in the vertebrate liver . . . I may express my conviction that this appearance of pigments like indigo carmine, carminate of ammonium, and Bismarck-brown in the granules of the walls of the alimentary canal in Polyzoa, taken in conjunction with the normal appearance in the same place of a natural pigment and the ultimate passage of much of that pigment into the brown body, is to be regarded as, in part at least, a process of excretion." I would suggest that *L. saltans* indicates that the function of excretion, so far as the alimentary canal is concerned, is concentrated in the expanded terminal part of the intestine, and that the yellow pigment of the liver diverticula is of a different nature. The cells of the rectum which produce the excretory pigment have an utterly different character to those of the liver diverticula. In fig. 22 the former, and in fig. 24 the latter type of cell may be seen. In the rectal excretory cell the nuclei are deep down and horizontally placed, as so often occurs in the secretory cells, and the granules appear to be forming near the nucleus and passing outwards towards the

vacuole, which is clear and obvious. In the liver-cell there is no vacuole at all, the nucleus is large and spherical, rather less deeply placed, and the granules appear to be forming in the inner or more superficial part of the cells and to be accumulating towards the base. The difference in appearance seems to me to indicate the physiological difference between katabolic and anabolic metabolism.

The curious pagoda-like arrangement of cells seen at the sides of the body of *L. loxalina* (fig. 6, *k.*) is probably excretory, and may perhaps be comparable in structure to the excretory organs described by Harmer for *L. crassicauda*, but the histological detail is insufficiently good for me to determine its exact nature. If it is so, the position would indicate an external opening on the side of the body just below the posterior lophophoral ectodermic gland and not on the epistome as in *L. crassicauda*, which is a considerable difference of position. It may be that the type of excretory organ described here for *L. saltans* and in others, as, for instance, *L. Davenporti* by Nickerson, is derived from such a condition as the above, but it might have been correlated more closely with such cells as those known as leucocytes in the Ectoprocta, which have been shown by Harmer to have an excretory function.

Reproductive Organs.—I have seen no trace of a hermaphrodite condition such as Nickerson describes for *L. Davenporti*, and which, I think, may occur in *L. loxalina*, though it is not possible to say that one individual may not produce ova at one time and sperm at another, as Harmer has suggested. It is possible that of my nine specimens of this species none was fully mature, but I do not think so. I find also that the gonad is single and median. In no instance have I seen a trace of a paired gland in either sex. Fig. 14 passes through the ovarian follicle of an individual which contains what appears to be a nearly fully grown oöcyte. The follicle opens by a narrow duct into the atrium between the intestine and the epistome (fig. 9, *g. p.*). The oviducts project so as to form a little papilla, which is seen cut across in fig. 9. I cannot find

any other organ which is undoubtedly connected with the function of reproduction in the females. There is nothing which I could compare with Nickerson's mammary gland, unless the slightly modified epithelium on the inner surface of the epistome may indicate a function of this nature (fig. 9, *m. e.*).

The male organs seem to me to be as simple as the female organs. There is a single follicle with duct opening on a papilla in the same place into the atrium, namely, between the intestine and epistome. The follicle contains spermatozoa instead of an oöcyte, and there is no modification of the external epithelium of the atrium.

I am not convinced that there is any glandular mass which can be compared with the so-called shell-gland of *L. Davenporti* and *L. annelidicola* or *L. loxalina*. So it will be seen that the reproductive system is as simple as possible, and remarkably different from the condition in other species of *Loxosoma*.

Body-wall.—The general parenchyma presents no special features. It is very sparse in *L. saltans*, and is dense where it occurs. In *L. loxalina* it is more abundant and less dense. There is a complete absence of any lateral expansions into alæ characteristic of *L. Davenporti* and *L. annelidicola*. The epidermis is distinct and a single layer of cells throughout, but varies, as we have seen, in different localities, giving rise to the peculiar "flask organs," the rows of large cells in the stalk and the supporting structure on the foot. The lophophore is drawn out into the sixteen tentacles, the structure of which is as follows: There is a central single row of dense cells, fig. 18 (*mes.*), round which are about five rows of ectodermal cells. The two outer rows are sharply defined, lightly staining cells, while on the inner face are three rows of larger cells with less well-defined boundaries. These three rows bear long stiff cilia which vibrate vigorously in the living specimen from time to time. On the outer surface there are one or two single stiff non-vibratile hairs, probably sense-hairs comparable to those described by Harmer

on *L. crassicauda*, and shown by Prouho on *L. annelidicola*. On the four large oral tentacles there is a little tuft of hair on the tip of each on its outer border as shown in fig. 10. These are the ones which seem to aid in locomotion.

Budding.—I have had so few specimens that I have been unable to study the development of buds. In no case did I find more than two buds; nor were these buds very large. It would be rash to say that two buds is the limit of the number borne at once by an individual, seeing that in other forms so many may be carried at the same time. On the other hand, it is not improbable that two should be the limit in this species, for, as one may well imagine, a little species like this which is capable of such active locomotion might not find it advantageous to be encumbered by more than a couple of buds at a time.

The only other *Loxosoma* that I have found in Scotland is *L. phascolosomatum* in the Kyles of Bute.

Two new species of *Loxosoma* have recently been described by Nilus in the 'Trav. nat. C.R. séances St. Petersburg,' vol. xl, and named *L. murmanica* and *L. Brumpti* respectively. These are very unlike the two species described in the above, and resemble *L. tethyæ* more closely, having a small circular lophophore with only six tentacles, with no very evident epidermic gland-organs, and with many buds arising low down on the body-wall.

#### SUMMARY.

(1) *Loxosoma loxalina* n.s.—Lophophore larger than the body and bears sixteen tentacles, of which four are longer than the others. These four are on the oral part of the lophophore, which is slightly inflected along that region. The stalk is considerably longer than the calyx, and terminates in a circular foot with radiating supporting cells. There is no foot-gland. The body and the lophophore are beset with deeply placed ectodermal organs along the lateral margins,



of which organs there are usually four pairs, giving a somewhat angular appearance to the calyx. It was found living commensally with a Maldanid worm (which was not identified) on the Morven shore of the Sound of Mull in Scotland.

(2) *Loxosoma saltans* n.s.—Lophophore larger than the body and bears sixteen tentacles, of which four are longer than the others and are moved separately, and are always outside and over the others when contracted. They bear stiff hairs on the outer edge of their tips. As in *L. loxalina* the oral part of the lophophore is indented. The specific name indicates its habit of locomotion by jumping, in which action the four large tentacles take a part. The stalk is shorter, or only slightly longer than the calyx, and terminates in a circular foot as in *L. loxalina*. There is no foot-gland. The glandular diverticula of the alimentary canal are highly developed. The body has two ectodermal organs, or less, which are pedunculate and attached to the ventro-lateral surface just below the buds. They are larger and placed higher than the corresponding organs of *L. Davenporti*. The species was found in the tube of a Maldanid worm *Clymene ebiensis* on the Skye shore of the Kyle of Loch Alsh in Scotland.

(3) The alimentary canal of *L. saltans* is differentiated more markedly into glandular, absorptive and excretory regions than *L. loxalina*. Two very distinct proximal diverticula appear to secrete some substance into the cavity of the gut, probably digestive in action; a more distal pair of diverticula seem to be more of the nature of a liver. The whole alimentary canal is a single layer of cells.

(4) The nervous system consists of the usual pair of ganglia in both species. In *L. saltans* the main nerves can be traced to lophophore, body-wall, kidneys and gut. Sensory hairs occur on the tentacles. A chain of cells along the stalk may be nervous.

(5) In *L. saltans* the excretory organs are divided into (i) lophophoral and (ii) body-kidneys, which are large vacuolated cells which perhaps lie in contact with ducts, but the external

openings could not be traced. No sign of ciliation of any part of these ducts is apparent in the living *L. saltans*.

In *L. loxalina* the excretory organs are on a rather different plan.

In *L. saltans* the (iii) rectum takes an important part in excretion, certain cells accumulating excretory products in vacuoles which presumably burst into the cavity of the rectum.

(6) In *L. loxalina* the reproductive glands consist of a pair of gonads which may be hermaphrodite, with ducts joining in the median plane where there is a shell-gland, whence a median duct runs to open into the atrium between the epistome and the lophophoral hood.

In *L. saltans* the gonad is single and median, with the duct opening on a papilla between the epistome and lophophoral hood.

(7) In neither species is there any lateral expansion of the body into alæ. The general mesodermal tissue is more abundant in *L. loxalina* than in *L. saltans*.

I desire to express my sincere thanks to Professor W. C. McIntosh, LL.D., F.R.S., for his kindness in identifying the Maldanid worm upon which *L. saltans* was found; to Dr. G. F. Harmer, F.R.S., for certain references to the literature of *Loxosoma*; to Miss Marie Krull for assistance in the preparation of the specimens; and to Miss Dorothy Thursby Pelham for drawings numbered 4, 5 and 6 upon Pls. 6 and 7.

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## EXPLANATION OF PLATES 6 AND 7.

Illustrating Mr. Richard Assheton's paper on "Loxosoma loxalina and Loxosoma saltans—Two New Species."

## LETTERING.

*a.* Atrium. *b.* Bud. *b. k.* Body-kidney. *c. c.* Ciliated cell of stomach region. *ec. ex.* External ectoderm of tentacles. *ec. o.* Ectodermal spike or flask-like organs of *L. saltans*. *ec. or.* Ectodermal organ of *L. loxalina*. *ect.* Ectoderm. *ect. ii.* Ectodermal organs of *L. loxalina*. *ect. iv.* Ectodermal organ of many cells deeply placed of *L. loxalina*. *ep.* Epistome. *ex. r.* Excretory cells of rectum. *ex. gr.* Excretory granules in rectal cell. *d. l.* Dorsal row of large ectodermal cells (nervous?). *gl. c.* Gland-cell (or supporting rod?). *g.* gonad. *g. d.* Duct of gonad. *g. p.* Genital papilla. *int.* Intestine. *k.* Kidney. *k. d.* Kidney-duct. *l. d.* Liver diverticulum. *l. k.* Lophophoral kidney. *m. e.* Modified epithelium of atrium. *mes.* Mesodermal core of tentacle. *m.* Mouth. *mu.* Mucous cell. *n. g.* Nerve-ganglia. *æs.* Œsophagus. *o. f.* Ovarian follicle. *oöc.* Oöcyte. *p. c.* Cell of pancreatic diverticulum. *p. d.* "Pancreatic" diverticulum. *r.* Rectum. *sh. gl.* Shell-gland. *st.* Stomach. *ten.* Tentacle. *tes.?* Testis?. *v.* Ventral wall of rectum, ciliated. *vac.* Vacuole in rectal cell. *vac.'* Granule entering vacuole.

Fig. 1.—*Loxosoma loxalina*. Preserved specimen seen from the ventral surface. The character of the alimentary canal and the position of the gonad and excretory organs (?), the nerve ganglia and ectodermal organs in their most characteristic form are indicated.

Fig. 2.—*L. loxalina*. Vertical longitudinal section of a corresponding specimen showing the mouth, alimentary canal and anus, which is placed near the edge of the lophophore.

Fig. 3.—*L. loxalina*.—Transverse section through the brain, æsophagus and intestine. One of the ectodermal organs has been cut and alongside of it the group of cells which are arranged in tiers and are probably excretory cells. Part of the reproductive system, probably the shell-gland, lies between the brain and æsophagus.

Fig. 4.—*L. loxalina*. A section taken transversely so as to cut the intestine (*int.*), the gonad (*g.*) and the edge of the brain (*n. g.*). A portion of the gonoduct is seen at *g. d.* running towards a group of granular cells (*sh. g.*), which are probably shell-gland.

Fig. 5.—*L. loxalina*. A vertical section through the epistome showing the opening of two ducts. One on the epistome may be excretory

the other one (*g. d.*) is a duct which leads from the group of cells of figs. 3 and 4 to the space between the epistome and the lophophore.

Fig. 6.—*L. loxalina*. A section taken horizontally through a gonad (*g.*) and a curious row of cells (*k.*), which are probably not connected with the gonad, but are of the nature of an excretory organ. These large vacuolated cells bear some resemblance to those of the excretory nephridium of *L. crassicauda* (v. Harmer).

Fig. 7.—*L. saltans*. Horizontal section cutting through the epistome and lophophoral kidney. The two fine kidney ducts are seen cut obliquely as they pass towards the epistome.

Fig. 8.—*L. saltans*. A section through the lophophoral kidney, which is seen to consist of several large, much vacuolated cells closely pressed together and bounded by a capsule.

Fig. 9.—*L. saltans*. Transverse section through the atrium passing through œsophagus, intestine and papilla upon which the oviduct opens.

Fig. 10.—*L. saltans*. A figure drawn from a living specimen to show the way in which the lophophore and tentacles are carried. The four long tentacles usually work together. The fine stiff hairs, used either as touch sense-cells or grasping organs, are seen on these four tentacles. The specimen bore one well-developed bud, one rudimentary one, and two of the peculiar "flask"-like ectodermic organs of unknown function. One of these is shown in the figure.

Fig. 11.—*L. saltans*.—A slightly oblique section which passes vertically through the œsophagus, one nerve ganglion, one "liver" diverticulum, but misses the rectum. The character of the liver-cells containing many granules is seen.

Fig. 12.—*L. saltans*. A horizontal section through the pancreatic diverticulum and stomach. The cells of the wall of the stomach are really ciliated, except the mucus-producing cells (*mu.*), but not the cells of the glandular diverticulum.

Fig. 13.—*L. loxalina*. Part of the foot shows the large rod-like cells which project beyond the general margin as either adhesive or supporting elements.

Fig. 14.—*Loxosoma saltans*. Transverse section through the brain, œsophagus, and intestine, the brain consisting of two ganglia connected by a thick band of nerve-fibre. Between the brain and œsophagus lies the ovary, consisting of a single follicle containing a single oöcyte. On each side the body-kidney is seen in section.

Fig. 15.—*L. saltans*. A section taken through the base of one of the pedunculate ectodermal organs, which consists of four modified ectodermal cells contained within a capsule of ordinary cells.

Fig. 16.—*L. saltans*. A section of the second on the same animal showing the ectoderm cut through on one side. These organs appear to be easily lost.

Fig. 17.—*L. loxalina*. Diagram of similar ectodermal organs on the species *L. loxalina*, in which they are never pedunculate. (a) The organ next the stalk; (b) any of the others.

Fig. 18.—*L. saltans*. Sections which illustrate the structure of the tentacles. Each consists of a core of mesodermal cells, a single row, and a coat of ectoderm of which the innermost are ciliated. Sense-hairs are borne by some of the outer cells, but these are not shown in the figure.

Fig. 19.—*L. saltans*. A figure drawn from a living specimen with tentacles retracted, showing the way the four large ones bend over the smaller lateral ones. The colour of the various parts of the alimentary canal are shown. The kidneys are also very slightly yellow, but these are not shown in the drawing.

Fig. 20.—*L. saltans*. A figure of a living specimen after twenty-four hours in sea-water containing a weak solution of methylene-blue. The parts brightly coloured are the young growing tissues, especially the ectoderm of the buds, the central nervous system, and the lateral walls of the rectum.

Fig. 21.—*L. saltans*. Another specimen which had been in sea-water with methylene-blue for some hours. The coloured parts are the kidneys, the rectum (also excretory), and a chain of cells along the dorsal wall of the stalk.

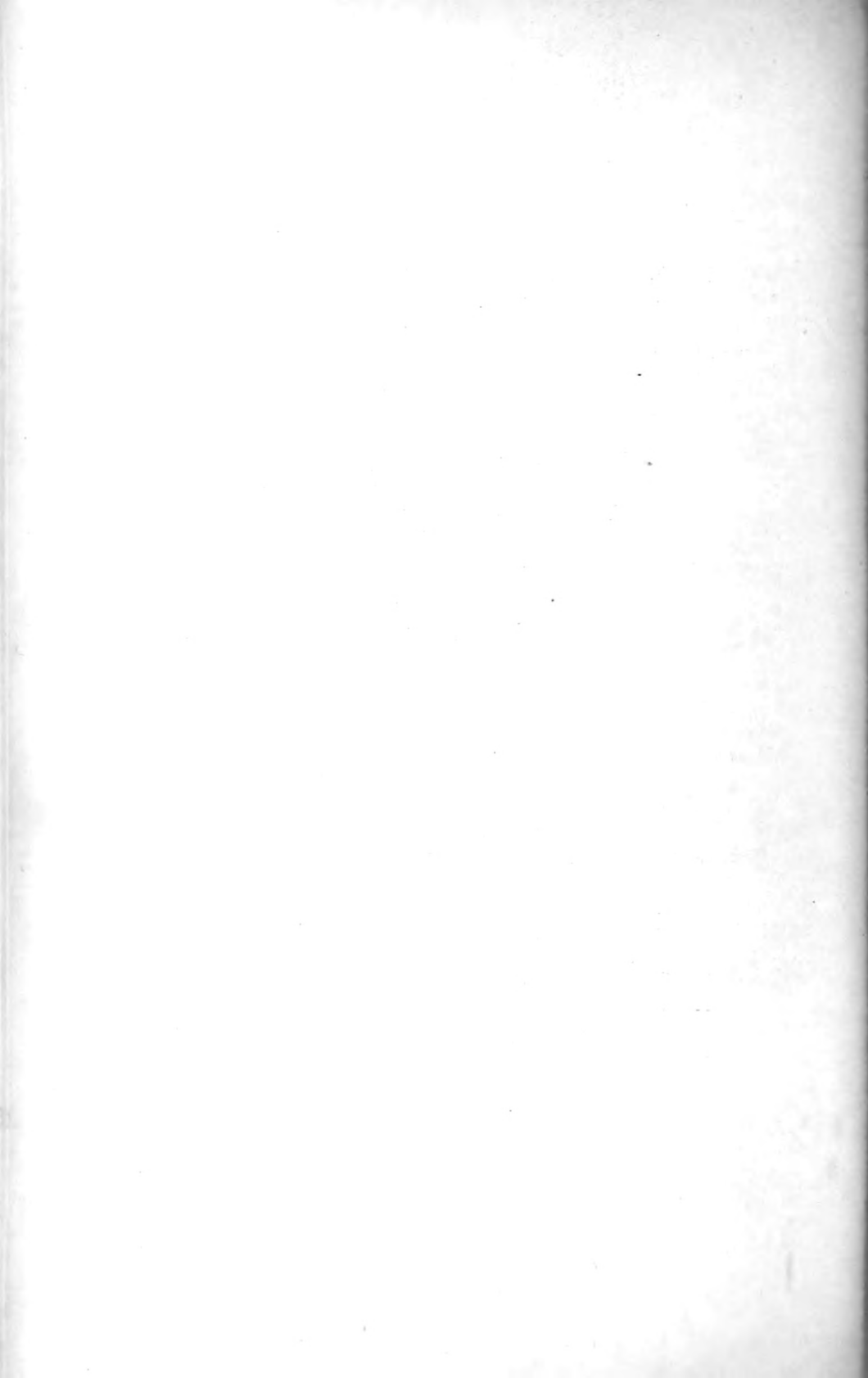
Fig. 22.—*L. saltans*. Two cells of the liver-like diverticula or thickening of the alimentary canal.

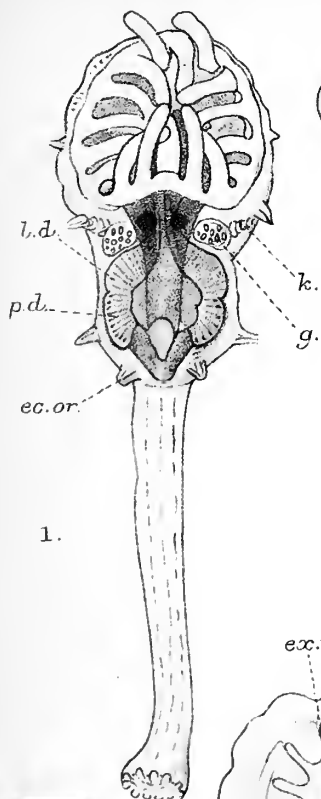
Fig. 23.—*L. saltans*. Two cells of the pancreatic diverticulum of the gut, and one ciliated cell of the "stomach" region.

Fig. 24.—*L. saltans*. A section through the rectum stained with thionin, orange G, and eosin. The dorsal and ventral walls are ciliated. The latter walls are composed of excretory cells. The excretory products are seen collecting in the large vacuoles.

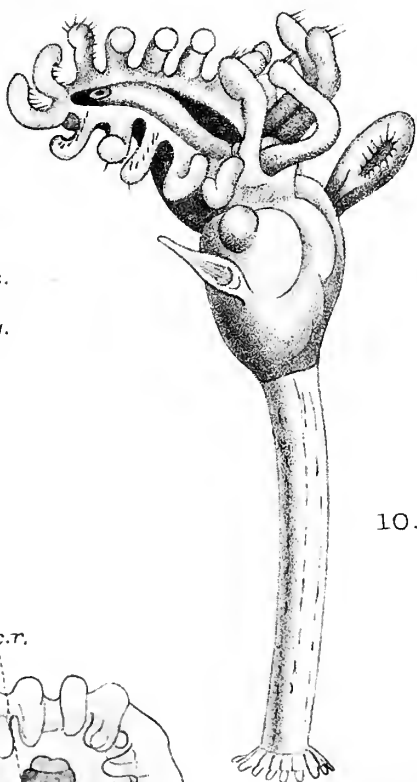
NOTE.—The specimens of *L. loxalina* were all fixed in Perenyi. Those of *L. saltans* in Flemming's or Hermann's fluid.



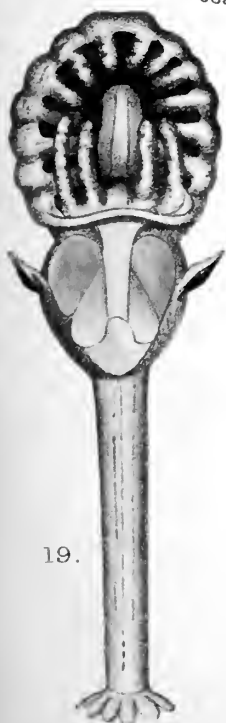




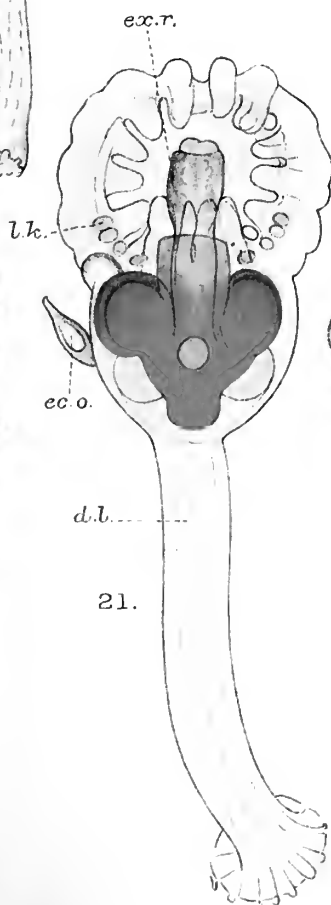
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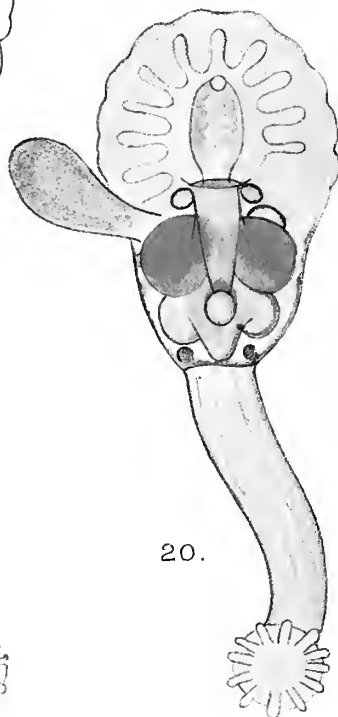
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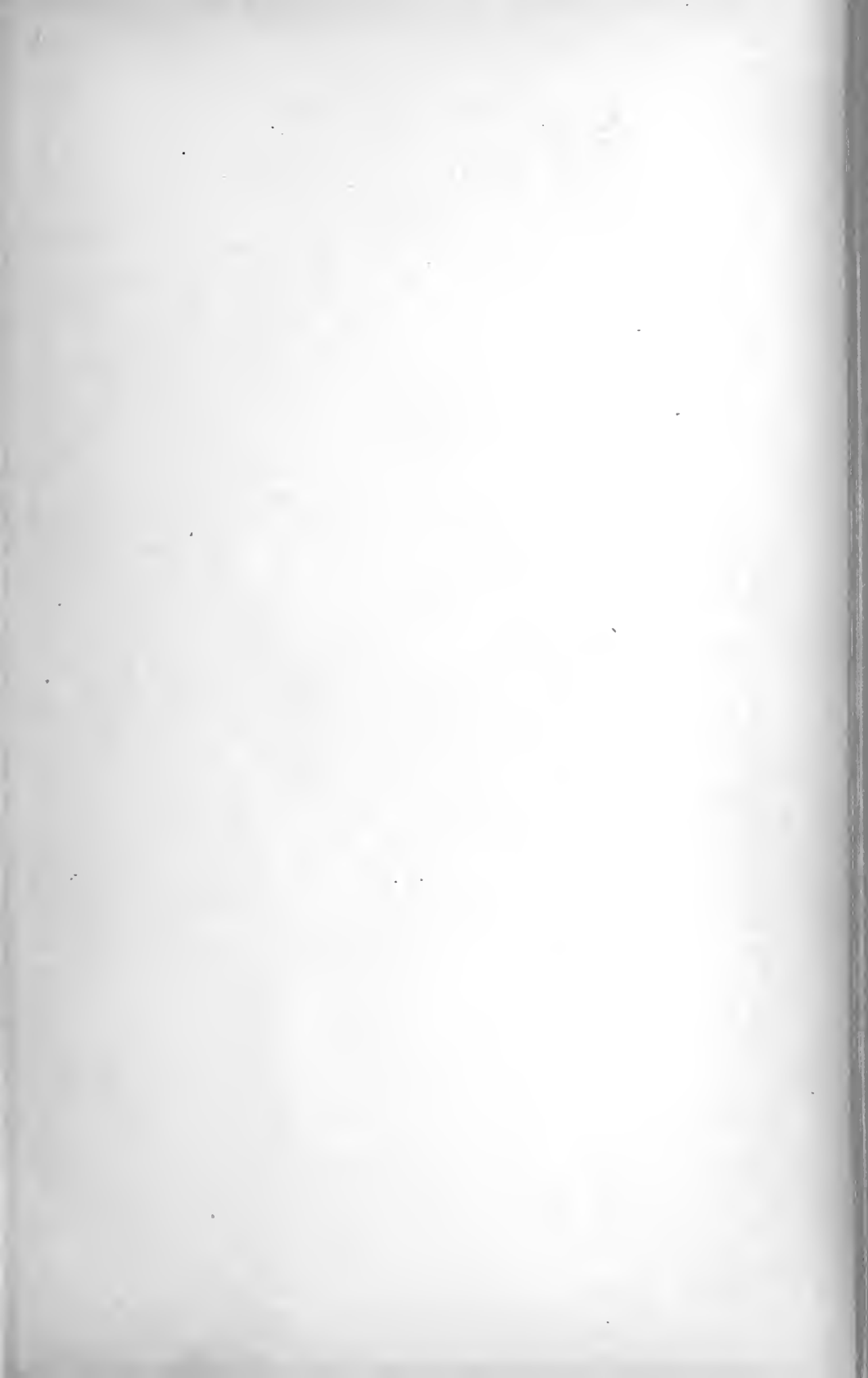


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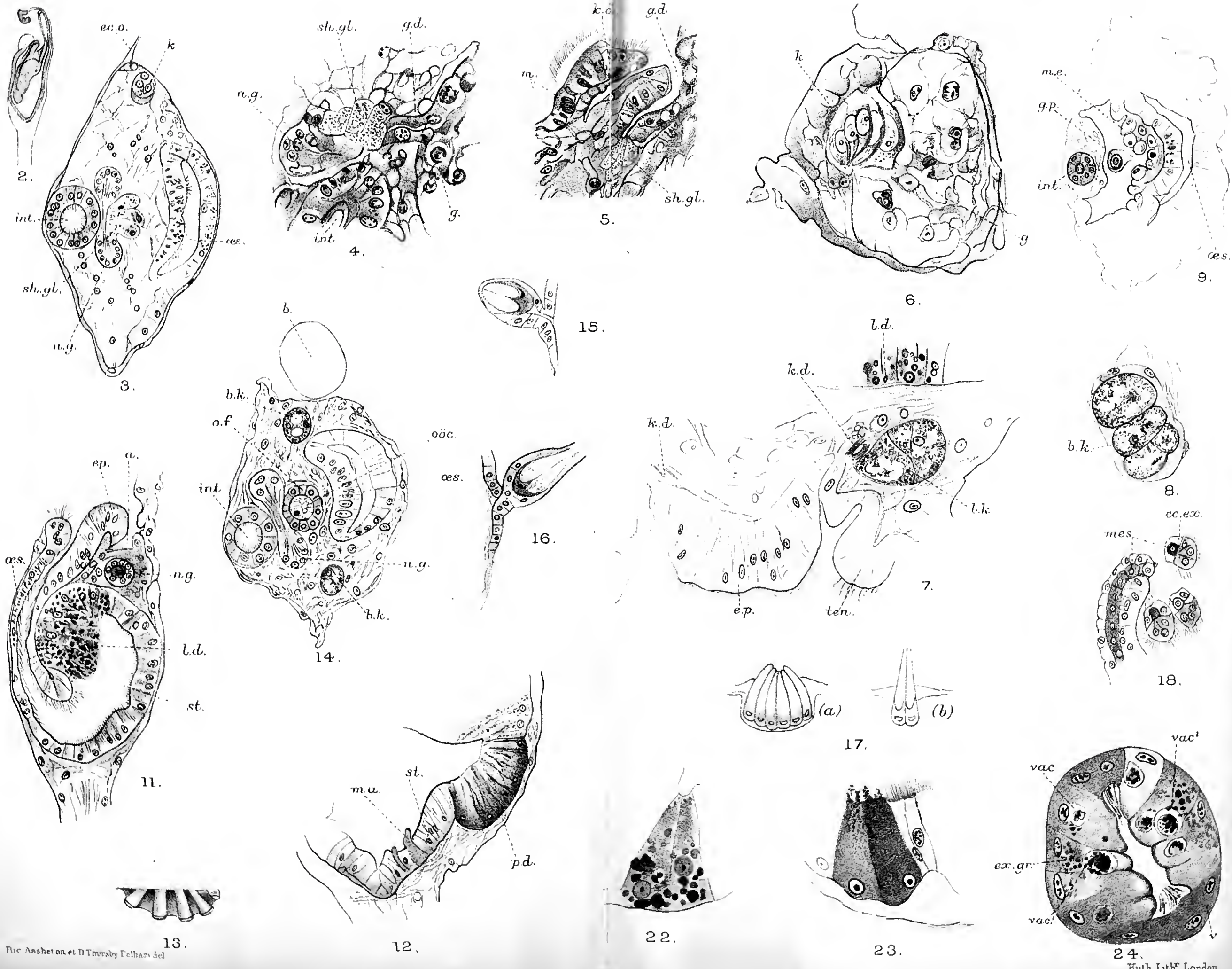


20.













## Gastrulation in Birds.

By

**Richard Assheton, M.A.,**

Lecturer on Physiology in Guy's Hospital, University of London.

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IN the twentieth volume of the 'Journal of Morphology' a paper by Mr. J. T. Patterson appeared during the year 1909 under the title, "Gastrulation in the Pigeon's Egg: A Morphological and Experimental Study," a preliminary notice of which was published in 1907 in the 'Biological Bulletin,' vol. xiii.

In these papers the author gave an entirely novel account of the process of gastrulation in a bird, which account, if free from error, described an interesting, albeit perplexing, phenomenon.

The paper is fully illustrated by photographs and diagrams, and has the appearance of being a careful piece of work, and it has been used by Professor Frank R. Lillie as the basis of his description of the early stages of bird development in his recent book, 'The Development of the Chick.' Professor Lillie writes, on page 52, that he "has had the opportunity of following the work step by step, and is convinced of its accuracy."

The paper describes so unusual a process that, in spite of this testimony, it courts a rather close examination. Moreover, if correct the matter should be relieved of all suspicion, because it would in that case be a highly important contribution to the embryology of birds.

Since it seems to me that the description given by  
VOL. 58, PART 1.—NEW SERIES.

Patterson is not altogether free from doubt, I venture to offer the following notes by way of criticism, which may possibly, and I hope will be, successfully met. Briefly stated Patterson's account is as follows: He denies that gastrulation in the pigeon's egg occurs by delamination or any process of ingrowth from the germinal wall or other lower layer segments. Gastrulation, according to him, takes place before the egg is laid by a process of involution of the outermost layer of cells of the segmented blastodisc. At the close of segmentation this outermost layer of cells, which forms a continuous membrane, becomes detached from the sub-lying cells and yolk along that part of its margin which is towards the future posterior end, and the detached margin becoming involuted, grows forward as a thin free edge beneath all the loose cells which admittedly exist in the deeper parts of the segmented blastodisc. This free edge joins up in front and at the sides with the wall of yolk that contains nuclei (i.e. the germinal wall), and forms a continuous sheet of cells—the entoderm or hypoblast. As the subgerminal cavity expands it excavates the germinal wall, and a sheet of cells derived from the germinal wall is left above the cavity. To this sheet the “invaginated” entoderm fuses. Thus the gut entoderm is formed by involution and the yolk sac entoderm by excavation. The loose cells lying beneath the outer layer, now to be termed “epiblast,” are said to pass into the outer layer, and so also to form part of the epiblast. This involution process is said to be still further complicated by the concrescence of the lip thus formed giving rise to a linear seam—the future primitive streak, which is withdrawn later within the area pellucida by a sweeping round of the germinal wall in a manner reminiscent of Duval's attempt to prove a process of concrescence at a time subsequent to the laying of the egg.

#### CRITICAL NOTES.

It is claimed that this account of the formation of the entoderm or hypoblast by an infolding of the blastoderm

edge is supported by experimental observations, such as marking certain parts of the blastoderm by injury, and following such marks through several hours of incubation.

Although the account given forms a very complete story, which, if the observations are good, does seem to be supported by a good deal of evidence, yet it is very difficult to reconcile it with the process of gastrulation in the other Amniota. In fact Patterson himself hardly mentions the reptiles or mammals, but confines his efforts to an attempt to adapt the bird to Amphibians and fishes, and more especially to the Teleostean fishes. That is to say, he tries to connect birds with a group far removed from them in anatomical features, and ignores the difficulties presented by his theory when compared with the most closely allied forms.

In no other group of vertebrates is the dorsal lip of the blastopore, which is in every other case the most bulky and actively proliferating part of the embryo, known to exist as a thin or free edge! It is extremely difficult to conceive of the mechanism by means of which such an involution could take place.

Again, on pp. 86-87, the author speaks of the whole thin edge as the dorsal lip of the blastopore, and the yolk as the ventral lip. Now this is never the case in any vertebrate, whether we consider the meroblastic eggs of the Elasmobranch or Teleost or the less heavily yolked eggs of the Amphibia. In all cases the ventral lip of the blastopore, if formed, is a thickened curved rim which is formed in conjunction with the inflection of the epiblast. If Patterson is right in calling the inflected edge of the blastoderm the dorsal lip of the blastopore, then the part which he calls the ventral lip of the blastopore is surely the floor of the gut corresponding to the yolk-plug in *Rana*. The yolk is never the lip of the blastopore.

Stronger evidence is the table given (p. 90) of measurements made upon living blastoderms during the time supposed to be taken for the process of gastrulation. If the edge of the ectoderm is inflected, one might expect to find a

diminution of the length of the blastoderm occurring at that moment. This is said to have been so in two eggs which were kept under observation for  $3\frac{1}{2}$  and  $3\frac{1}{4}$  hours respectively.

Patterson objects to some experiments made by myself in 1896 ('Proc. Roy. Soc.,' vol. lx, 1896) with a view to testing Duval's theory of conerescence on chicks, thus: (1) They were performed after conerescence had occurred, which is a valid objection if Patterson's contention that all this occurs before laying is correct. (2) The cells may have flowed round the bristle held in place by vitelline membrane and yolk.

This objection, which, of course, is irrelevant in this particular case if the first holds good, must be considered as a general objection to the use of bristles for such purposes. The bristle, it may be said, makes a perfectly unmistakable landmark, which cannot be said of injuries by canterisation.

The objection is one which has naturally occurred to me, but I am convinced that the objection is groundless for the following reasons:

In the numerous experiments made upon chick and frogs' eggs with sable hairs, I have never seen any evidence that cells can flow round the hair.

The results would not be so constant if there were any flowing of cells round the bristle.

If cells could move so easily as to avoid a bristle without making any visible sign of disturbance, they would be affected by the force of gravity and become displaced when eggs are not in their normal position. This is not the case. A fully segmented egg of *Rana temporaria* may be held down at an angle of  $90^\circ$  to its normal position without affecting the normal relation of its cells to one another. When there has been a very severe drag upon the bristle on account of some excessive stress in an egg, due to some displacement with reference to the vitelline membrane, and an attempt has been made by the cells to flow round the bristle, the effect is obvious, and is seen as a bay or wrinkle which is quite absent from properly performed experiments of the kind, and indicates only an attempt of a soft tissue to swing round the

obstacle, and is not in any way comparable to an actual flowing of a fluid past a fixed and solid object.<sup>1</sup>

If the segmented egg of a frog, or the segmented blastodisc of a bird, were perfect fluids, then the objection would be a fatal one. Or if the segmented egg were like a heap of shot, then also such a mass could flow slowly past a fixed object without producing any visible rippling. But the segmented ovum is not a pile of separated cells. The cells, or many of them, are in continuity by means of viscous cytoplasmic strands, and the whole mass is by no means a perfect fluid.

If a bristle is inserted into the yolk-plug of an egg of *Rana temporaria* during the crescent stage of the blastopore close up against the advancing dorsal lip of the blastopore, there is no tendency either for the advancing lip to be divided by the bristle, nor for the bristle to be driven through the yolk plug-cells, thus proving the absence of anything approaching a perfect fluidity of either the ectodermal or the endodermal layer of cells.

I may remark here that when one of my experiments suits Patterson's purpose he accepts it! (p. 115). If cells can sweep past to congregate when the bristle is placed to one side, the fact that my bristle, when placed in the area opaca in the posterior margin, did not appear in the embryo, is no proof that the cells that do form the embryo have not come sweeping past the bristle.

Personally I cannot agree with Patterson's view (p. 109) that conrescence and gastrulation are different phases of the same process. Gastrulation is the formation of the gut cavity. If this formation is accompanied by the production of a blastopore (which is by no means always the case, e.g. Hydrozoa, probably all mammals—I would even add all

<sup>1</sup> These remarks refer to *Rana temporaria* only. There is much variation in the viscosity of amphibian eggs. I have failed with the segmented egg of *Triton cristata*. The eggs of *Bufo* are also less suitable than those of *R. temporaria* owing to greater fluidity. The results obtained from the chick and *Rana temporaria* I believe to be quite reliable.



Amniota), then that blastopore may close by concrescence, but the two processes are entirely different phenomena.

I have myself tried for years to emphasise this difference ('94, '96, '08, '09), and the difference is recognised by many embryologists such as Hertwig, Hnbrecht, Keibel, MacBride, although they do not use the terms which I humbly protest do most correctly express the essence of the process, namely protogenesis and deutero-genesis. The phenomenon of gastrulation or the formation of the primitive gut-cavity or archenteron, whether with or without a blastopore, is protogenetic, and represents a more ancient phase of evolution. The subsequent phenomenon of deutero-genesis is growth in length and is post-gastrula, and in those animals which have a blastopore formed in connection with the first appearance of gut-cavity it involves all the changes by which the blastopore becomes wholly or partially closed, whether by coalescence, convergence, or concrescence, partial or total. It represents a stage in evolution subsequent to that represented by the gastrula stage.

If there is any concrescence it is concerned with deutero-genesis in the vertebrates and not with gastrulation; but it is extremely doubtful, in spite of Patterson's work, whether there is any such thing as concrescence in the sense which can be interpreted as meaning that the embryo of the vertebrate is formed by the fusion of the lips of an elongated blastopore.

Patterson adheres with patriotic tenacity to the view so commonly held by Americans as to the formation of the vertebrate embryo by concrescence. He writes thus on p. 103: "In other words, in the teleost the entire margin of the blastoderm separates from the periblast, and this entire margin (germ-ring) concresces to form the embryo." He was presumably unaware of Kopsch's work on the eggs of *Salmo*, 1905, or he could not possibly have written so dogmatically. Kopsch's experiments prove as conclusively (so it seems to me) as anything can be proved that in the trout the main dorsal axis of the embryo is not formed by concrescence.

From these experiments it is perfectly plain that the germ ring representing the lips of a posteriorly placed blastopore provides the material for growth in length thus—the mid-dorsal part for the mid-dorsal region of the embryo, the nerve-cord and notochord, the lateral parts for the sides, the ventral part for the ventral surfaces. I may refer the reader to some remarks on this in my paper on Teleostean development, 'Guy's Hospital Reports,' vol. lxi, 1907.

If, therefore, Patterson's account of the formation of the main axis of the pigeon by concrescence is correct it is interesting and remarkable, but at any rate it is not like the Teleostean.

Again, where in the animals most closely connected with the birds in adult characters, the reptiles and mammals, can we possibly find the slightest hint of any process either of an involution of a free edge or a process of concrescence?

If we turn from such general considerations to his actual experiments we are not convinced by them.

In the first place there is some, but not much, chance of mistake in the orientation. Patterson says that in the pigeon's egg the embryo lies with its longitudinal axis at an angle of  $45^{\circ}$  with the longitudinal axis of the egg ("chalazal axis") in 90 per cent. of eggs. Presumably he discarded experiments in which on the development of the embryo it was found to deviate from  $45^{\circ}$ .

Exp. I. (Operation  $33\frac{1}{2}$  hours, examination 37 hours after the estimated time of fertilisation.)

The posterior margin of the blastoderm, at this time a free edge, was injured by cauterisation before it had become involuted, which injury "ought to be carried down beneath the blastoderm during the course of further development, that is, it ought to be found in the entoderm" (p. 88).

The truth of this contention is supposed to be demonstrated by a photograph (fig. 66). There is nothing to indicate which is anterior or posterior end, but I take it that the number "66" is close to where the edge of the blastoderm should be, and that the space under the letters "op" represents the deficiency in the entoderm. We are asked to compare

this with a section of "an uninjured blastoderm at a corresponding stage," and to note that "the entoderm in this region is very thick (see fig. 37). It is clear, therefore, that while such an operation destroys most of the cells that are to give rise to the entoderm, yet the posterior margin is still capable of forming a rounded dorsal lip." I venture to submit that it is perfectly impossible to deduce any such conclusion from the figures given.

Fig. 66 represents a magnification of 125 diameters, and the point of injury is about  $3\frac{1}{2}$  in. from the dorsal lip. Fig. 37 is magnified 245 times, but as the whole section measures less than 5 in. it cannot contain the required spot. There is, however, another figure of the same section, fig. 35, which is magnified 107 times. If we examine the region  $2\frac{1}{2}$  in. or even 2 in. to the left of the edge of the blastoderm, we fail to see any greater accumulation of entoderm cells in the uninjured than in the injured one.

Possibly I may have made a mistake in my interpretation of his fig. 66, and the number "66" is at the anterior end and not the posterior end as I assumed. In that case I am at a loss to find either the cells which have been injured or the deficiency in the entoderm referred to. If the latter is indicated by the clearer spot near a letter "z" (of the figure above) then the corresponding spot in fig. 35 or 37 is just as devoid of entoderm as in 66. Or if, as he seems to suggest, we are to contrast fig. 67 with a part still further to the left in fig. 35, I fail to see much difference in the condition of the "entoderm." On this latter assumption, the spot labelled "op" is presumably the "break" in the vitelline membrane made by the operating needle, from which the free edge has curled away forwards. Since there is not a trace of vitelline membrane shown, the photograph fails to strengthen the argument in the text.

I think it must be admitted that the author has not been successful here in his attempt at demonstration.

Exp. II. (Operation  $35\frac{3}{4}$  hours after fertilisation. Subsequent incubation 49 hours.)

The injury was again on the edge, but now the edge is a lip. In these he finds an injury in entoderm only, therefore he says there is still an inrolling of entoderm.

In specimens "slightly older" such experiments show injuries in ectoderm and mesoderm but not in the entoderm, "showing that the involution has ceased."

The whole series of experiments recorded under the heading "Experiment II" seems to me to be questionable in the extreme. Anyone reading the first two paragraphs of that section with a critical mind must perceive how fragile is the evidence upon which such far-reaching results are based. He writes (p. 93), in describing the subsequent effect of an injury made to the edge of the lip in the middle dorsal line, "There is no evidence of an injury either in the ectoderm or mesoderm, and hence we must conclude that the affected cells have been brought to their present position (in the entoderm) by an inrolling under the posterior margin. Although this operation has been repeated several times with the above results, yet the position of the injury in the entoderm may vary in an anterior posterior direction; but this variation is easily accounted for by the fact that one can tell in the living egg only approximately the extent to which invagination has progressed."

Thus we see the results obtained are variable; and he goes on to say that "if an injury be made in the same manner as above on slightly older blastoderms, the affected region is not found in the entoderm, but in the ectoderm and mesoderm, showing that the involution has ceased" (p. 93).

There is little here in the nature of exact or accurate experiment. There are no times<sup>1</sup> or measurements given, but instead of these he bases results upon operations performed on "slightly older" blastoderms than those the stage of development of which "one can tell only approximately."

There is also the difficulty presented by this hypothesis of formation of a blastopore lip before the laying of the egg,

<sup>1</sup> Some times are given in the explanation of the plates.

that there would then be a very striking difference compared with other vertebrates. In all other vertebrates the blastopore lip is the growing point for growth in length, and growth in length begins at once, therefore showing itself clearly in the origin of denterogenetic (peristomial) mesoderm from the angle of the lip laterally, notochord dorsally, and deuterogenetic epiblast superficially. This condition is well known not to occur until some hours later—in the chick about the twelfth to fifteenth hour of incubation. So we should have to account for a very remarkable disappearance and reappearance of this proliferating centre.

Again, Patterson in his second series of experiments says that an injury made during the involution process is found in the entoderm, posterior to the position of the nineteenth pair of mesoblastic somites. We are faced with the following dilemma. We can hardly have a proliferating blastopore lip formed as in other vertebrates so long as the outer layer is turning in to form entoderm. Therefore this proliferating lip must come into being after the cessation of that process. Any injuries made to the involuting membrane must surely occur in front of, or beyond, all the tissues, mesoderm included, which are produced by the proliferating lip when it comes into being.

But fig. 50 shows such an alleged injury far posterior to the nineteenth pair of somites. The injury ought to be in front of all the primitive streak mesoblast, whereas, according to the figure, there are many somites of mesoblast in front of the injury.

Another argument which is difficult to follow is the suggestion on p. 99 that certain "cavities in the dorsal lip" are the homologues of Kupffer's vesicle. It is surely well enough established that whatever the physiological meaning may be of Kupffer's vesicle in Teleostean development, it is, as a cavity, part of the gut-cavity. According to Patterson the archenteron is the cavity roofed in by the inturning edge of the blastoderm. Yet here he says that these vacuoles above this roof are homologous to the Kupffer vesicles, which are

well known to be below this roof, i. e. they are part of the archenteron.

Exp. III. (Operation  $34\frac{1}{3}$  hours after fertilisation. Subsequent incubation 34 hours.)

From the plan of his text-fig. 16 one is bound to conclude that the stage does not materially differ from the stage of the preceding experiment, his text-fig. 10. Each shows a similar diameter, a similar forward extension of the endoderm, a similar width of what he regards as blastopore opening. The only difference is that in text-fig. 10 the dorsal lip of the "blastopore" is slightly convex, in text-fig. 16 slightly concave in surface view.

In Exp. II an injury was made on the edge, and the result was a defect in the endoderm at a spot posterior to the nineteenth somite.

In Exp. III an injury was made on the surface just within the margin. The difference in position of the injury would appear to be not more than the diameter of the needle used. Result, a defect in the region of the head-fold. Therefore the difference in position of less than a needle's diameter in the marking of a blastoderm corresponds with a difference in the embryo which includes the greater part of the body. If this is so we must despair of getting anything approaching accurate results by such methods.

Patterson likewise thinks it unlikely that this small area should give rise to so much embryo directly, and assumes, as we have seen, that there is a conrescence.

Exp. IV. (Operation  $34\frac{2}{3}$  hours after fertilisation. Subsequent incubation for  $36\frac{1}{4}$  hours.)

On a blastoderm similar to Exp. III a spot was marked on the margin  $10^\circ$  to the right of the middle line so close to the margin that the outer surface of the needle was level with it. Result, a defect "on the right neural fold in the mid-brain region."

If the main axis of the embryo is formed by coalescence of the two germ-rings, surely, then, it is in the median plane that the injury should be found, i. e. the ventral wall of the neural



tube and notochord and, perhaps, gut, yet the defect is shown on the upper part of the neural tube only.

Exp. V. (Operation  $33\frac{1}{3}$  hours after fertilisation, subsequent incubation for  $36\frac{3}{4}$  hours.)

A similar blastoderm was injured at the edge of the horn of the junction zone  $45^\circ$  to the right of the middle line, with the result that a defect is said to have occurred in the primitive streak, though one cannot see much of it in his fig. 71.

In none of the cases so far considered do the figures convince one that the spots called defects are really such, or have any constant relation to the spots injured.

For instance, in the last case it is quite impossible to satisfy oneself that there is any injury at all from fig. 71, and text-fig. 17, which is a transverse section through the alleged injury, shows a perfectly normal primitive streak section with a mass of cells or yolk, or both, lying on the top in no way connected with it. This, in fact, is an "extra-ovate" in Ronx's sense that may have travelled from anywhere. My own experiences with such experiments have taught me how deceptive an extra-ovate may be.

The results are very different to the defects figured by Kopsch in his Sahnö embryo experiments.

Exp. VI. At a rather later stage—"late gastrular stage"—in which the entoderm had advanced a little further an injury was made at the posterior margin in the median line.

The result was a defect in the middle line at the level of the tenth pair of somites affecting ectoderm only.

Exp. II was supposed to demonstrate the involution of the edge of the blastodisc to form the entoderm, because an injury to the edge made at  $35\frac{1}{4}$  hours appeared only in the entoderm somewhat posterior to the nineteenth pair of somites. In Exp. VI an injury also touching the edge although made three quarters of an hour earlier appeared in the ectoderm only. How can this discrepancy be explained away on Patterson's hypothesis?

Now this one seems open to another explanation. There is clearly an extra-ovate consisting of "a mass of dead cells"

lying between the separated halves of the neural tube. The notochord is perfect and to one side, the entoderm is uninjured. May not the defect in the neural tube be simply mechanical, due to the pressure of an extra-ovate which became separated off from the edge of the blastoderm as a result of the canterising, and which, passing into the area pellucida between the blastoderm and vitelline membrane, caused the injury seen? Text-fig. 19 strongly suggests this solution.

Exp. VII and VIII. The figures do not enable one to appreciate the character of the defects. Sections are not given.

The remaining experiments, IX-XIII, were made upon the blastoderm after the eggs were laid, and therefore after Patterson's supposed concrescence of the lip must have been completed, because by now, according to him, the main axial line of the embryo produced by this concrescence is entirely enclosed within the blastoderm margin, and there are no longer any free blastoporic lips that could come together.

Although Patterson still speaks of concrescence, Exp. XI, p. 115, it clearly cannot be a phenomenon similar to that which, as he alleges, occurs during gastrulation. One is inclined in this particular connection to say with Professor MacBride (re "*Amphioxus*," *Quart. Journ. Micr. Sci.*, vol. liv, p. 302): "Of course in every structure there is an imaginary middle line, and if anyone chooses to say that this band of dividing cells consists of right and left halves which unite together as quickly as they grow, I shall not waste time in arguing against such a metaphysical conception, which is capable neither of proof nor disproof."

From Patterson's final discussion it is clear that he quite fails to appreciate the distinction between gastrulation and subsequent growth in length. It is not really true that "all of the chorda and mesoderm are derived from the primary invaginated layer" in *Amphioxus*. The anterior part is so derived, but the posterior part is derived from the proliferating lips of the blastopore, which can be described neither as ectoderm nor endoderm.

To me it seems that there is a very great difference between gastral and peristomal mesoblast: the one is protogenetic, the other deuterogenetic.

Nor, again, is it true that in the case of birds the whole mesoblast is formed from the primitive streak. It is altogether difficult to understand why if the primitive streak is formed by the fusion of thickened rims, that thickening should disappear, only to reappear a little later as primitive streak. It is very remarkable and significant of the narrowness of this work that in dealing with avine early stages as compared with other vertebrates the word "reptile" should occur only twice and the "mammal" is mentioned but a single time!

It is pretty evident that the author is utterly unable to reconcile his description (which is an attempt to fit the birds on to fishes) with the facts of reptilian or mammalian embryology, the two groups of animals most nearly connected with the birds.

## Studies in the Experimental Analysis of Sex.

### Part 9.—On Spermatogenesis and the Formation of Giant Spermatozoa in Hybrid Pigeons.

By

**Geoffrey Smith, M.A.,**

Fellow of New College, Oxford.

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With Plate 8.

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THE cytological investigation which forms the subject of this study is based on the examination of the testes of three male hybrids, which resulted from the crossing of a female domestic dove with a male magpie pigeon. The three birds which were the sole progeny of this mating were all males, and I may mention here that the only other hybrid bird I have succeeded in obtaining was also a male, the cross in this case being between a male bantam and a female pheasant. Of about sixty eggs incubated from this latter cross only one chick was obtained, which suffered from a cerebral hernia and died two days after hatching. The testes of this hybrid were perfectly normal, and microscopical investigation showed they were in the same state of development as in an ordinary chick of the same age. The fact that all the four hybrid birds dealt with by me were males is in complete agreement with Guyer's records, which show an enormous preponderance of males over females in hybrid pigeons and gallinaceous birds (1).

Two of the three hybrid pigeons (A and B) which furnished

the material for this study were of a uniform slaty-blue colour, with a white bar on the ends of the tail-feathers which was particularly conspicuous when the birds were flying; the third (C) in its plumage took more after the dove-parent, though the dove-colour was darkened by a slaty tinge. This bird also had the white tail-bar, but was otherwise unmarked.

The birds lived in my aviary for more than a year; they actively courted the female pigeons put in with them, and two of them (hybrids A and B) were successfully paired off with two females, which laid eggs. The hybrid in each case assisted in the process of incubation, in the way usual for male pigeons, but in both cases the eggs were infertile and came to nothing.

The hybrids were killed in June, 1911, and dissection showed that all three had apparently normal genitalia, with very large testes, normal vesiculæ seminales containing large quantities of spermatozoa, and normal cloacal papillæ.

The spermatozoa examined alive under the microscope were seen to be in active movement, though some of them were obviously deformed, possessing bead-like thickenings along the course of their heads. Slides of the spermatozoa preserved with corrosive sublimate and stained with iron-haematoxylin were obtained, and sections of the testes preserved and stained in the same manner were prepared. For the sake of comparison similar preparations of spermatozoa and of testes sections were made from ordinary male pigeons and from male domestic doves.

An examination of the smear preparations of the spermatozoa of the hybrids, as compared with the spermatozoa of the normal male pigeon and dove, brought out the remarkable fact that besides the hybrid spermatozoa being often deformed, the great majority of the undeformed spermatozoa from all three hybrids are about twice as long as the spermatozoa from the normal males of either pigeon or dove. A reference to Pl. 8, figs. 1-5, representing camera tracings of the spermatozoa, will help to bring out this fact. It will be seen that the size of the heads of the spermatozoa from

the normal male pigeon and dove (figs. 1 and 2) vary within narrow limits, and that the heads are straight and of a fairly uniform thickness throughout. In the case of the three hybrids (figs. 3-5), the spermatozoa, drawn to the same scale, are seen in the majority of cases to be about twice the length of the normal spermatozoa, while some of them have thickened beads of chromatin along their length and are obviously deformed.

A few of the hybrid spermatozoa drawn in the figures are of about normal size, but they are only few compared to the large or deformed varieties. In considering the size of the spermatozoa, only the heads are taken into account, as the tails are so delicate and stain with such difficulty that it was found impossible to deal with them.

As it was desirable to establish what proportion of the hybrid spermatozoa were twice the normal size, camera tracings were made of a large number of spermatozoa taken at random from the normal and hybrid males, and the camera tracings were measured with dividers.

Spermatozoa showing beads upon them or complex coils were not used, only the comparatively straight and uniformly thick heads being measured. The samples of the hybrid spermatozoa were therefore not perfectly random samples, but since it is the large spermatozoa which usually exhibit the beads and coils, the error introduced would tend to diminish the proportion of large to small spermatozoa.

As to the method of measuring the camera tracings with dividers, it is obvious that a rather large experimental error is introduced owing to the spermatozoa heads being curved, but by carefully stepping with the dividers over the sharper curves and not attempting to take account of variations less than a millimetre, the main results may be relied upon.

The result of the measurements of the spermatozoa heads of the three hybrids and of three normal male pigeons and of a normal male dove are given in the subjoined table, the frequency with which the various sizes of spermatozoa heads occur in each case being read off in the horizontal columns.



TABLE I.

Size in mm. $\times$ 550.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	Totals
Normal pigeon 1	6	150	25	—	—	—	—	—	—	—	—	—	181
Normal pigeon 2	6	70	40	8	—	—	—	—	—	—	—	—	124
Normal pigeon 3	1	38	50	10	—	—	—	—	—	—	—	—	99
Normal dove .	1	23	51	24	3	—	—	—	—	—	—	—	102
Hybrid A . .	1	6	9	6	1	5	7	13	19	17	3	1	88
Hybrid B . .	—	—	—	—	4	5	9	25	29	5	2	—	79
Hybrid C . .	1	2	3	2	2	5	6	14	32	13	3	1	84
Sum of hybrids .	2	8	12	8	7	15	22	52	80	35	8	2	251
Sum of normals .	14	281	166	42	3	—	—	—	—	—	—	—	506

The table shows the frequency distribution of sizes of random samples of spermatozoa heads from normal and hybrid birds. The modal value for the normals lies between 6 and 7; for the hybrids, between 12 and 13.

It will be seen that whereas the spermatozoa of the normal males vary from 5-9 (to get the actual measurement in mm. these figures must be divided by 550), with the modal value between 6 and 7, the spermatozoa of the hybrids vary from 5-16 with the modal value between 12 and 13; in other words, the majority of the hybrid spermatozoa are just twice the size of the normal. In the last two horizontal columns the frequency of the various sizes of all the hybrids summed together and of all the normals is given, and if we regard all the hybrid spermatozoa measuring from 11-16 as being double the normal size, then 79 per cent. of the hybrid spermatozoa may be regarded as double the normal size. This is probably an under-estimate, owing to the reason already given, viz. that the beaded and twisted spermatozoa which are not included are practically all of the double size, and they constitute about 50 per cent. of the total number.

Having established this curious abnormality of the hybrid spermatozoa in point of size, the next step was to inquire whether any explanation of it could be found in the processes of spermatogenesis in the hybrid testes.

Professor M. F. Guyer published in 1900 an interesting paper (2) on spermatogenesis of normal and hybrid pigeons, and he described the occurrence of beaded spermatozoa in the sterile hybrids similar to those which I have found and figured, but he does not allude to any definite abnormality of size in the spermatozoa except in a few cases. With regard to spermatogenesis, Guyer describes the normal process in ordinary male pigeons, and also the process in his hybrids, and he found various abnormalities in the maturation divisions of the latter, especially in the first maturation division, these abnormalities consisting in the frequent formation of multipolar spindles, and in irregularities of the synaptic process. His conclusion is that there is something repellant in the two germ-plasms which go to make the hybrid, of such a kind that the synaptic chromosomes which ought to fuse together before the reduction division repel one another or fuse abnormally, and that this condition upsets the normal equilibrium of the cell, so that it divides atypically.

The result of my own observations is to confirm Guyer's idea substantially, and to supplement his account with certain positive details which give a complete explanation of the occurrence of the giant spermatozoa we have described, and also throw a strong light on the cause of the sterility of these hybrids.

The process of maturation in the normal male pigeon and dove may be first described, but only the main points need be considered here, as my observations agree with those of Guyer in every particular (2). Figs. 6-8 on Pl. 8 show the important stages in the normal pigeon and dove. The primary spermatocyte in the first maturation division shows eight rounded chromosomes arranged regularly on the mitotic spindle, these chromosomes being approximately equal in size. There is no evidence of the existence of an odd or accessory chromosome at this or any other stage of the process. The chromosomes which appear on this mitotic spindle have often the appearance of rings. There can be no doubt

that each chromosome is bivalent, and represents two chromosomes joined together in synapsis, since about sixteen chromosomes can be counted in the earlier spermatogonial divisions. On the completion of the first maturation division it seems that the second division is immediately begun.

In the second division, instead of eight chromosomes appearing on the equatorial plate, four large chromosomes appear of the same size as in the preceding division. There can be very little doubt that these chromosomes are again bivalent, and that consequently a second synapsis of some kind normally takes place in birds. This double synapsis was described for the pigeon by Guyer in 1900, and it has been observed by him since in several other species of birds (3 and 4). Presumably this second reduction division is only a pseudo-reduction, and does not involve another halving of the chromosomes. After the second maturation division is completed, the resulting cells pass into a resting phase (figs. 6*c* and 7*c*) as spermatids. The transformation of these spermatids into spermatozoa takes place in the usual way by the elongation of the cell-body with its nucleus, the chromatin in the nucleus becoming drawn out into a spirally twisted thread (figs. 7*d* and 8). In transverse sections, under a high power, the ripening spermatids have the appearance shown in figs. 6*d* and 7*d*, where the chromatin thread is seen to lie up against the nuclear membrane.

To compare this normal process with what occurs in the hybrids, reference must be made to the figures 9, 10 and 11, Pl. 8, and these figures should be compared with figs. 6, 7 and 8. The mitoses in the hybrid cells all represent the first maturation division, and it will be seen at once that in the first place the chromosomes are not arranged regularly on the mitotic spindle; secondly, that they are of very unequal and irregular sizes and that they are more numerous than in the normal spermatocytes. It is evident that a normal synapsis to form eight similar bivalent chromosomes does not occur in the hybrids; the separate chromosomes do not come together and fuse in an orderly way, but are scattered irregu-

larly over the spindle and distributed apparently at random to either end of the spindle in the division. Synapses apparently do occur between certain chromosomes or even groups of chromosomes, but the whole process is plainly abnormal. I have not observed any cases of multipolar mitoses such as Guyer describes, but otherwise in the inequality of size and distribution of the chromosomes our observations agree.

But now occurs the remarkable process which accounts for the hybrid spermatozoa being of double normal size. After a careful search through the material of all three hybrids I can find no trace of the second maturation division ever occurring at all. Instead of this the secondary spermatocytes are at once converted into spermatids of twice the normal size, which proceed forthwith to transform themselves into spermatozoa. Many of these spermatids are of normal appearance (figs. 9 *b* and *c* and 11 *b* and *c*), except that their nuclei and cell bodies are just twice the normal size, and they become elongated in the ordinary way to form the normal double-sized spermatozoa. Others, however (figs. 10 *b* and 11 *d*), appear abnormal from the first, quite apart from their large size, the nucleus being uniformly stained a deep black with hæmatoxylin, and it is evident that these give rise to the abnormally twisted, branched or beaded forms of spermatozoa, some of which are figured on Pl. 8, figs. 3-5.

The process of transformation of these abnormal spermatids into deformed spermatozoa is shown in some cases in figs. 10 *b* and 11 *d*. The fact that the second maturation division is suppressed accounts completely for the double size of the majority of the hybrid spermatozoa; the occurrence, however, of a comparatively small percentage of small or normal-sized spermatozoa in the hybrids leads one to suppose that the second division does occasionally take place, though so rarely that it would easily be missed.

The point at which the spermatogenesis in the hybrid begins to become abnormal is at the first maturation or reduction division. A comparison of the spermatogonia of the normal

and hybrid birds has shown that in point of size and histological structure they are indistinguishable; the first sign that anything is wrong appears in the mitotic spindle of the first maturation division, where the normal eight synaptic chromosomes are not formed. Instead of this, irregular masses of chromatin are distributed to the opposite poles of the spindle, so that the secondary spermatocytes formed as the result of this division do not receive a normal heritage of chromosomes. It appears that the disturbance caused by this abnormal distribution of the chromosomes prevents the second division taking place, the secondary spermatocytes, instead of dividing, going straight on to form spermatids and spermatozoa. Many of the spermatozoa so produced are greatly deformed, as is not unnatural; others, which have presumably received a more normal supply of chromatic material, give rise to spermatozoa which are normal in appearance, though double the normal size. But even those spermatozoa which are normal in appearance are impotent in fertilisation, and we must again ascribe this to the fact that they do not contain the right numbers or kinds of chromosomes. The observations detailed above afford strong support to Guyer's view that the principle cause of the sterility of hybrids is the fact that the two sets of parental chromosomes, although they may co-operate together in the ordinary cells of the hybrid, yet when they have to go through the process of synapsis, repel one another or form abnormal fusions, with the result that the germ-cells so produced, though they may appear normal, are constitutionally abnormal. This explanation of the sterility of hybrids can only apply to those hybrids which succeed in producing germ-cells of some sort, or at any rate get as far as the maturation divisions; it may fail to explain the complete sterility of females, for instance, in which the eggs fail to grow or store yolk, since in these cases the abnormality of the reproductive cells manifests itself before synapsis occurs.<sup>1</sup>

<sup>1</sup> This difficulty, however, may be more apparent than real, since von Jenkinson informs me that a precocious synapsis is known to

It is possible, therefore, that the immature reproductive cells of hybrids may be affected by the fact of their hybridity more readily than the ordinary somatic cells, and that this sensitiveness may exist independently of the occurrence of synapsis and the reduction division. But this does not detract from the importance of the cases we have been considering, where everything was normal in the hybrid until it came to the first reduction division.

Theoretically, the facts brought out by Guyer and by this paper emphasise the fundamental importance of synapsis and the reduction division, and support the view that this process is a vital one in the orderly sorting out of the properties of the two germ-plasms derived from the two parents.

A further theoretical consideration is suggested by the facts. The reproductive organs and even a large number of the spermatozoa of these sterile hybrids had the outward appearance of being normal, and yet, according to our interpretation, the spermatozoa were impotent owing to the abnormal distribution of the chromosomes to them. Is it not possible that the sterility of certain individuals within any particular species may be due to similar causes; that there may exist intra-specific hybrids, so to speak, which, owing to their combining in their germ-plasms incompatible qualities, are not capable of forming functional gametes? Again, it is possible that the disturbances in the expected Mendelian proportions which are known to occur so frequently in intra-specific crosses may be due to the impotence of particular gametes carrying particular characters. If this were so, if the combination of certain characters in a germ-plasm led to abnormal distribution of the chromosomes during maturation and to the consequent impotence of certain types of gamete, we would obtain what in effect would be tantamount to selective fertilisation. At any rate it is certain that some-

occur in many female animals at a very early stage, even in the embryo, long before the oocytes enlarge. If this is the case in birds we obtain a ready explanation of the complete sterility and abortion of the ovary in hybrid females, and possibly also of the high death-rate of hybrid females.



thing more than simple segregation is required to obtain the complicated results at present vaguely included under the term "Mendelian inheritance," and it may be suggested that the cytological evidence obtained from the study of these hybrids indicates the kind of physiological basis which may underlie some of these peculiar Mendelian results.

#### SUMMARY.

(1) The study is based on the cytological investigation of the spermatozoa and of spermatogenesis in normal male pigeons and doves, and in three male hybrids produced by the mating of a male pigeon with a female domestic dove.

(2) The ripe spermatozoa of the hybrids, which were present in large quantities, besides showing in certain cases structural abnormalities, were on the average twice as large as the normal spermatozoa of either parental type.

(3) The first maturation or reduction division in the hybrids is abnormal, in that the chromosomes do not enter into the normal synapse to produce eight synaptic or bivalent chromosomes, but they are scattered as irregular chromatic masses of unequal size on the mitotic spindle, and are irregularly distributed to the opposite poles of the spindle.

(4) The second maturation division in the hybrids is almost entirely suppressed, the secondary spermatocytes proceeding without further division to form spermatids and spermatozoa of twice the normal size. Many of these spermatozoa are structurally normal, apart from their double size, while others are abnormally twisted or beaded. All the spermatozoa were probably impotent, since these hybrids and all others of a similar kind are invariably sterile.

(5) The explanation of the sterility of such hybrids is found, in accordance with Guyer's idea, to reside in the disturbance of the synaptic division during maturation, this disturbance being due to the incapability of the chromosomes derived from the specifically different parents to fuse to form the normal synapses.

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  3. ——— “The Spermatogenesis of the Domestic Chicken,” ‘Anatomischer Anzeiger,’ Bd. xxxiv, 1909, p. 573.
  4. ——— “The Spermatogenesis of the Domestic Guinea,” ‘Anatomischer Anzeiger,’ Bd. xxxiv, 1909, p. 502.
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## EXPLANATION OF PLATE 8.

Illustrating Mr. Geoffrey Smith's paper on “Studies in the Experimental Analysis of Sex.”

[All the figures are drawn with the camera under a 4 eyepiece and a  $\frac{1}{12}$ -in. objective. Magnification about 1270. The tails of the spermatozoa are somewhat schematised, but the heads give accurate proportions. All preparations fixed in corrosive-acetic and stained with iron-haematoxylin.]

Fig. 1.—Spermatozoa of normal domestic dove.

Fig. 2.—Spermatozoa of normal pigeon.

Fig. 3.—Spermatozoa of hybrid A between pigeon ♂ and dove ♀.

Fig. 4.—Ditto of hybrid B.

Fig. 5.—Ditto of hybrid C.

Fig. 6.—Stages in spermatogenesis of normal pigeon. 6 *a*. First maturation division, showing about eight subequal chromosomes on equatorial plate. 6 *b*. Second maturation, showing four chromosomes. 6 *c*. Resting spermatids. 6 *d*. Maturing spermatids in transverse section with a nurse-cell or cell of Sertoli.

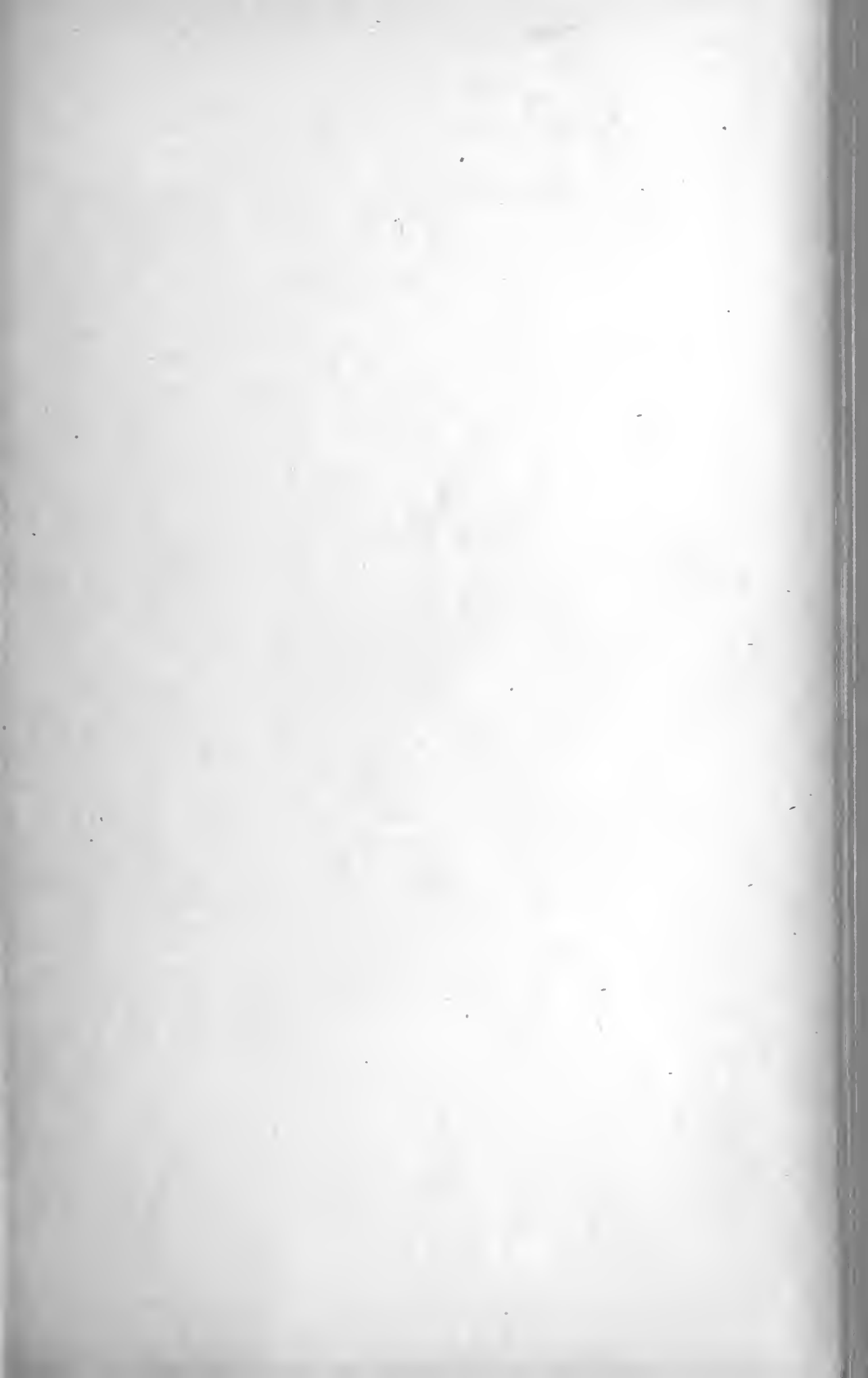
Fig. 7.—Spermatogenesis in another normal pigeon. 7 *a*. First maturation division. 7 *b*. Second maturation division. 7 *c*. Resting spermatids. 7 *d*. A nearly mature spermatid in longitudinal section, and two in transverse section.

Fig. 8.—Spermatogenesis in normal dove. Two first maturation divisions, one second maturation division, three resting spermatids, and three maturing spermatids.

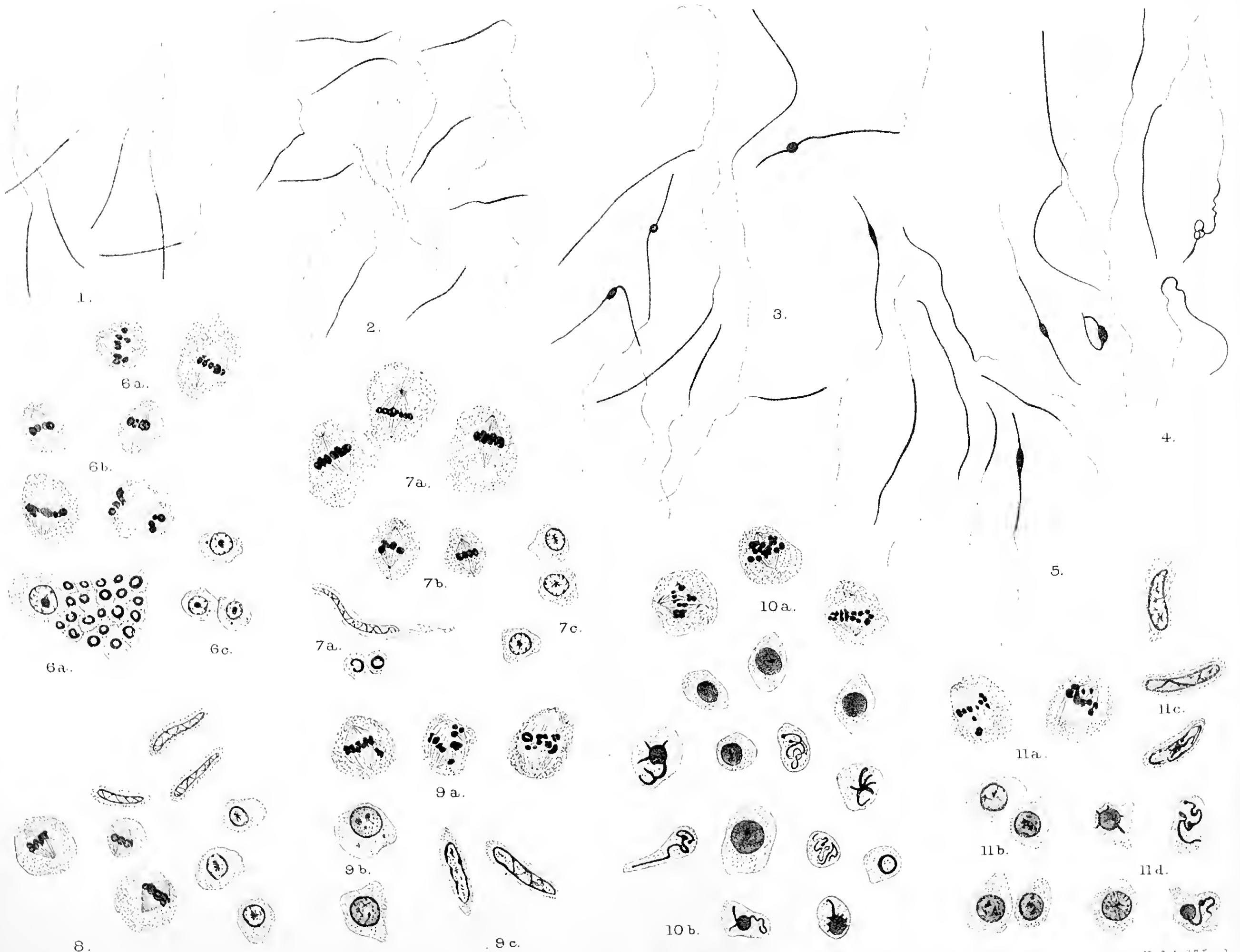
Fig. 9.—Spermatogenesis in hybrid A. 9 *a*. First maturation divisions, showing irregular and unequal chromosomes scattered on mitotic spindle. 9 *b*. Resting spermatids of double normal size. 9 *c*. Elongating spermatids of double size.

Fig. 10.—Spermatogenesis in hybrid B. 10 *a*. First maturation divisions. 10 *b*. A number of highly abnormal double-sized spermatids, some of which are being atypically converted into deformed spermatozoa.

Fig. 11.—Spermatogenesis in hybrid C. 11 *a*. Two first maturation divisions. 11 *b*. Five resting spermatids of double normal size, but otherwise normal. 11 *c*. Three elongated spermatids of double normal size, but structurally fairly normal. 11 *d*. Three highly abnormal spermatids in process of conversion into atypical and deformed spermatozoa.











Notes on Sporozoa. Nos. II, III, and IV.<sup>1</sup>

By

**H. M. Woodcock, D.Sc.,**

Lister Institute of Preventive Medicine, London.

With Plates 9 and 10.

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## PREFACE.

WHILE working at Rovigno during April and May, 1909, I examined a few of the common wall-lizards (*Lacerta muralis*), which occurred abundantly on the neighbouring islet of Figarola, as in the time when Prowazek studied the intestinal flagellates of this reptile (26). My object was really to see if a Trypanosome also occurred in it, Prowazek not having stated whether he examined the blood for that purpose or not; but I did not succeed in finding any Trypanosomes. In the blood-smears made from two individuals, however, a *Hæmogregarine* was found to be fairly plentiful. This *Hæmogregarine* is the same as that first described by Danilewsky (6) under the name *Hæmogregarina lacertæ*, and again later by Labbé (13), who placed it in a distinct genus, *Karyolysus*. I was too much occupied with other

<sup>1</sup> For the first of these Notes ("On *Klossiella muris*, Smith and Johnson"), vide 'Quart. Journ. Micr. Sci.,' vol. 48, 1904, p. 153.

work at the time to undertake a study of this parasite, so that I only made a few smears and cover-slip preparations from the blood.

Having an opportunity recently, I thought it might prove worth while to give some attention to these preparations, particularly to those stained by iron-hæmatoxylin, because—so far as I am aware—no observations have been made up to the present upon the nuclear structure of *Karyolysus*, as it is seen when the parasite is fixed and stained by the best cytological methods. My idea was, principally, to compare the nucleus of this *Hæmogregarina* with that of the piscine form (*Hæmogregarina rovigensis*) from *Trigla lineata*, an account of which has been given by Minchin and Woodcock (20). I had not long examined my preparations, however, before observing that a remarkable agreement was apparent between the nuclear condition of *Karyolysus* at a certain period of the life-cycle and that of a particular Coccidian in the corresponding phase. A study of the different forms of individual present in my smears has led me to the conclusion that they all belong to one species of parasite. This result has an important bearing, in my opinion, upon the question of the distinctness of many of the so-called species of Lacertilian *Hæmogregarina* which have been described, as I hope to show below. Lastly, the observation of the occurrence of a prominent karyosome, whose behaviour agrees closely with that of the characteristic coccidian karyosome, induced me to study again, from this point of view, the nuclear condition present in *Leucocytozoon* and *Halteridium*, as it is found in these parasites when fixed and stained in a similar manner.

## II. OBSERVATIONS ON *KARYOLYSUS LACERTÆ* (DANIL.), TOGETHER WITH REMARKS UPON THE SPECIFICITY OF THE *HÆMOGEGARINÆ* OF LIZARDS.

I will first give an account of *Karyolysus* as it occurs in my preparations. With two or three exceptions, all the

individuals observed are intra-cellular. In the blood of both the infected lizards the great majority of the parasites occur under one of two different aspects, which might lead one, at first sight, to conclude that two distinct species were concerned; but after a careful comparison of many individuals of both kinds, no doubt is left in my mind that they represent respectively early young phases and rather later, older forms of one and the same parasite. The two types of form (as they may be designated for the present) are distinguished chiefly by the position and character of the nucleus, which I will consider in detail presently; the latter feature can only be studied properly in preparations made by the "wet" method. I will merely say here that in the first type of individual the nucleus is situated more or less about the middle of the body (Pl. 9, figs. 2-8, 19-29), whereas in the other type it is close to one end (figs. 9-18, 30-40).

As regards their general appearance, both kinds of individual are usually bean-like in shape, the younger parasites being more slender and now and then slightly crescentic, the older ones broader and stouter. The individuals of both types vary somewhat in size, the former being, as might be expected, slightly shorter on the whole and distinctly narrower than the latter; but a few forms which possess the nuclear characters of the first type are met with, which are approximately as large as others possessing a nuclear arrangement of the second type. The dimensions of the younger forms, as seen on "wet" films, vary from  $8\ \mu$  by  $2\frac{1}{8}\ \mu$  (fig. 22) to  $9\frac{1}{2}\ \mu$  by  $2\frac{1}{2}\ \mu$  (fig. 26); those of the older individuals from  $9\ \mu$  by  $2\frac{1}{4}\ \mu$  (fig. 39) up to  $11\frac{1}{2}\ \mu$  by  $3\ \mu$  (fig. 33). On "dry" smears the larger parasites are probably rather flattened out; the extreme limits of variation in size (of either kind of individual) noticed are from  $11\ \mu$  by  $2\frac{1}{2}\ \mu$  (fig. 2) up to  $13\ \mu$  by  $4\frac{1}{2}\ \mu$  (fig. 17). The largest bean-shaped individuals, however, such as those of figs. 14-17, have undoubtedly acquired that appearance secondarily, by the lateral fusion of the two arms of a U-shaped form, the U-shaped form resulting in the first place from the further

growth and extension of the body-cytoplasm of a smaller individual. I have found different stages of the process in my preparations. The development of the U-form takes place only, so far as I have observed, in those parasites in which the nuclear position is that of the second type mentioned above. It begins by the formation of a small outgrowth at one end of the body, which is at once curved back and so extends backwards close along one side of the body (figs. 10, 36, 40). This outgrowth may arise either at the nuclear end of the parasite or at the opposite one, more usually, I think, at the latter. As it grows this process gradually forms one arm of the U, and at length the two arms become more or less equal (figs. 14, 15). Ultimately the two arms unite and a stont bean-shaped form results.

In nearly all of the individuals observed in "wet" preparations, of whichever type they may be, immediately surrounding the body of the parasite is a distinct space, which in some cases is very marked (cf. figs. 20, 22, 24-40). This space is probably due to the greater contraction of the parasite, as a result of the technique, than of the cytoplasm of the red blood-corpuscle enclosing it, thus causing a shrinkage of the former away from the latter. In ordinary "dry" smears, stained with Giemsa, this space is also often seen, though not so regularly as in the other preparations (cf. figs. 5-8, 12). In the case of the smaller parasites there is probably no definite membrane or envelope bordering the space on its outer side, distinct, that is to say, from the inner margin of the cytoplasm of the corpuscle (cf. for instance fig. 19, where the young *Hæmogregarine* has obviously just entered the host-cell). In the older (larger) forms, however, there is certainly a definite envelope present, constituting a delicate but firm capsule around the parasite (cf. especially figs. 37-40). In the case of two of the parasites figured it will be noticed there is no sign whatever of the cytoplasm of the blood-corpuscle; the reason for this will be mentioned shortly (pp. 177, 179). Hence the capsule surrounding the parasite is very conspicuous. In many cases where the cytoplasm of the

host-cell is still apparent the delicate capsule does not stand out in such a marked manner, but its presence is clearly indicated, in my opinion, by the following consideration. Many of the older forms, those, i. e., of the second type, in which the nucleus is situated near one end, appear very dark in "wet" preparations, being stained diffusely and more or less uniformly, so that it is very difficult to distinguish the nucleus. This appearance is really owing to the stain deposited inside the capsule or envelope not having been sufficiently extracted subsequently, the differentiating agent not having had time to penetrate properly inside the capsule, thus leaving the parasite overloaded with stain. I have never found this state of things, it should be noted, in the younger parasites, with the nucleus still near the middle; hence the capsule does not appear to be formed during the early phase. This capsule or envelope present in certain forms of *Karyolysus* appears to be very similar to that described in the case of small forms (young schizonts) of *Hæmogregarina triglæ* by Minchin and Woodcock (20). In the case of *Karyolysus*, however, I am inclined to think that the capsule is rather a definite envelope formed by the parasite than merely a sheath or altered layer of the cytoplasm of the blood-corpuscle (cytocyte), as we regarded it at the time in *H. triglæ*; its persistence and distinctness in such individuals as those drawn in figs. 37 and 38 supports this view.<sup>1</sup>

Considering now the nuclear structure in detail (as it is seen in "wet" preparations, stained with iron-hæmatoxylin), in the first type of individual, where the nucleus is situated near the centre of the parasite, the most striking feature is the very frequent occurrence of a conspicuous, deeply staining body, which is closely associated with the nucleus, lying at one side of it, contiguous to, but not actually forming part of, the general nuclear substance (figs. 19, 21-25). This latter consists, as in other *Hæmogregarines*, of a network containing small but fairly prominent grains of chromatin, most of which

<sup>1</sup> The mode of origin of the capsule may be really the same, of course, in *H. triglæ* also.



are usually disposed near the periphery. The limit or border of the nucleus is well-defined, but I am a little doubtful whether it can be regarded as constituting a true nuclear membrane. In some cases there are two of the above-mentioned conspicuous bodies, approximately at opposite sides of the nucleus (figs. 20, 26); these are generally unequal in size, neither being as a rule so large as when there is only one. Frequently these elements are seen to be surrounded by a very clear zone or halo (figs. 19, 20 and 25).

In the other type of individual there is usually no such large, deeply staining element associated with the nucleus (figs. 30, 32-36, 38-40). This contains fairly uniform grains of chromatin, which, on the whole, are distinctly more prominent and stain rather more deeply than those in the nucleus of the first type; now and again one of these grains is seen to be somewhat larger than the rest. Nevertheless, in a few instances, parasites belonging to this second type of form, with the nucleus near one extremity, do also show a large, deep-staining body in close association with the nucleus (figs. 31, 37), which is quite comparable to, or at least represents, that seen in the case of individuals of the other type. Much more frequently, however (though not always), in place of this element close to the nucleus there is noticeable a body lying at or near the surface of the protoplasm of the parasite, usually about the middle of its length (figs. 32-36). This structure may be nearly as large as that just described, but it is generally smaller, and may be very inconspicuous (fig. 36); where it is large it stains fairly intensely, but it is never so dark and black-looking as in the other cases, and, moreover, it has a much duller appearance and not such a well-defined outline.

I propose to leave for the moment the question of the significance of these bodies. It may be added that in Giemsa-stained smears on the other hand, in which the nucleus of the parasites generally appears to consist of large, irregular or ill defined masses of chromatic substance, it is only seldom possible to distinguish a more deeply staining element at

one side, which probably represents the characteristic element above described (cf. fig. 8).

The effects of the parasite on the host-cell are very pronounced and characteristic, as is well known to be the case, of course, in *Karyolysus*. The gradual alteration of the red blood-corpuscle and its appearance when infected by the different forms of parasite merit description, however, since this change is of great importance and assistance both in determining the relation to each other of the two chief types I have described, and also in connection with the question of the various species of *Hæmogregarina* (*Karyolysus*) said to occur in this lizard. The earliest change in the appearance of the host-cell which I have noticed is drawn in fig. 2 (from a Giemsa smear). The parasite infecting this corpuscle is one of the smallest observed, and has the nucleus centrally placed. Comparing the host-cell in this case with an ordinary uninfected red blood-corpuscle, its nucleus is found to be already distinctly larger, i.e. hypertrophied, but still oval in shape and not much elongated. The cytoplasm of the corpuscle is also slightly hypertrophied, but it is still stained to about the same degree and shade of colour as in an uninfected cell. This is, however, almost the only instance I have noticed where the cytoplasm appears stained similarly to what is the case in an uninfected cell. It is remarkable how quickly the presence of a *Karyolysus*-individual in a corpuscle produces some effect on the cytoplasm which results in a complete alteration of its staining properties. In nearly all the corpuscles infected with this *Hæmogregarina*, the cytoplasm has either taken up the stain only slightly, being faintly coloured, or else is very pale, practically unstained, so that it is often a matter of extreme difficulty to discern it at all. This is especially the case in wet preparations, stained by iron-hæmatoxylin; and in this respect *Karyolysus* differs markedly from certain other intra-cellular parasites of red blood-cells, of which I have preparations stained in a similar manner. For example, in *Hæmogregarina triglæ* (cf.

the figures of Minchin and Woodcock, loc. cit.) and again in *Halteridium noctuæ* (cf. below), the cytoplasm of an infected corpuscle is usually stained deeply, like that of an uninfected one, even where, as in the former case, there is a certain amount of hypertrophy; in these, the parasite appears as a clear space, almost vacuole-like, surrounded by the dark cytoplasm. In *Karyolysus* the appearance is quite different. Figs. 20-22, 24-26 represent early stages in an infection as early as, or slightly later, than that of fig. 2, from a Giemsa smear. The nucleus of the corpuscle is either oval or beginning to elongate. In such cases the cytoplasm can still be made out, but it never appears any darker than is indicated in Figs. 20, 22, 24. The nucleus also retains the stain much less intensely than in an uninfected cell stained by the same method (cf. fig. 23), the actual masses and grains of chromatin standing out sharply from the finely granular or reticular ground substance. The host-cell nucleus, it will be seen, is at once displaced by the parasite, and pushed to one of the longer sides of the corpuscle.

From being oval or slightly extended, the host-cell nucleus gradually becomes considerably elongated and greatly narrowed, i. e. compressed (figs. 32-40); all stages in this transition can be found, the real change in shape being best realised, of course, in preparations stained by iron-hæmatoxylin. In most cases the corpuscle-nucleus, in its final condition, appears like a slightly crescentic band, which is closely apposed to the parasite (or rather to its envelope) and follows its contour, curving round somewhat at either or both ends; this portion of the cell-nucleus is generally a little broader, i. e. less compressed than the rest, giving the whole nucleus the appearance of a bent club or halter, as the case may be.<sup>1</sup> In all these instances the axis of extension of the host-cell nucleus is approximately parallel to the length of the parasite. Now and again, however, where the corpuscle-

<sup>1</sup> The resemblance between this hypertrophied nucleus and that of the spindle-shaped host-cell infected by *Leucocytozoon* is often striking (cf. figs. 11, 12, 18 and 19, Pl. 10).

nucleus has either not been quite parallel to the longer axis of the cell to start with, or else has become twisted round somewhat by the entry of the parasite, the longer axis of the Karyolysus is more or less oblique to that of the host-cell nucleus, the one lying, as it were, across the other; in these cases, fission of the host-cell nucleus into two or more portions nearly always results (figs. 29, 11, 30). An important point must be mentioned regarding the appearance which one of these nuclei, in its final condition of hypertrophy, may occasionally present on a Giemsa smear, since it affords, I consider, another example of how the over-staining tendency of this stain may mislead and cause erroneous interpretations. In a few cases a mass of staining substance is seen, fitting like a cap round one end of the parasite, or there may be such a mass round both ends (figs. 16-18). These masses stain similarly to the nucleus of the host-cell, lying at one side of the parasite, and in fact may be distinctly connected with this and manifestly portions of it; it may happen, however, that such a mass appears almost or entirely separate from the nucleus, especially in flattened-out parts of the smear. Nevertheless there can be no doubt that these caps of staining substance represent also in such cases merely the wider, club-shaped end-ports of a crescentic host-cell nucleus, as above described, only here they are greatly overloaded with stain. These "caps," it is important to note, are distinctly on the outer side of the capsule enveloping the Hæmogregarine.

As indicated above, the cytoplasm of the infected corpuscle becomes ultimately so colourless that it is quite impossible to discern it (cf. figs. 37, 38 from wet preparations and fig. 17 from a Giemsa smear); in these cases it cannot be said whether it is still present or not.

The two forms of the parasite can now be considered in relation to the particular degree of alteration shown by the host-cells respectively infected by them. As a rule, in corpuscles which are in the earlier stages of alteration, with the nucleus still oval or only beginning to elongate, the parasites are of the first type described, with the nucleus

central and having the conspicuous, deeply staining body in close association with it (figs. 20-26). On the other hand parasites of the second type, with the nucleus near one end of the body, occur nearly always in corpuscles in which the alteration is far advanced, the cytoplasm being, at the best, only with difficulty discernible and the nucleus greatly elongated and narrowed (figs. 32-40). As is only to be expected, however, occasional exceptions to the above regular conditions are to be met with. Thus, an individual may be found, having its nuclear arrangement of the first type, which has already caused considerable elongation and alteration of the host-cell nucleus (figs. 8, 28, 29); conversely, a parasite may have acquired the second type of nuclear condition before the corpuscle-nucleus has become very elongated and narrow (figs. 10, 31). It may be regarded as practically certain, therefore, that the second type of individual is a rather later or older phase in the development of the first type of parasite. In addition to the evidence afforded by the various stages in the alteration of the infected corpuscles, this conclusion is also supported by the following points. Parasites of the second type are on the whole distinctly larger, that is to say, they have more bulk than those belonging to the first category; further, the only individuals seen free (fig. 21), or which have manifestly only recently entered a corpuscle (figs. 19, 26), have the first type of nuclear arrangement.<sup>1</sup>

It is not difficult, I think, from a careful comparison of different individuals, to form a fairly accurate idea of the manner in which the change in nuclear position and character is brought about; and for this purpose it is necessary to study the behaviour, in relation to the nucleus, of the characteristic deep-staining body which is associated with the latter in the young forms of the *Hæmogregarine*. In the earliest phase this body, which from now onwards I will designate according

<sup>1</sup> I have never observed any individuals of the larger, older type free—that is to say, which could have been liberated from a corpuscle, whether with or without the enveloping capsule.

to its true significance, namely, as a karyosome, is single and relatively large; it is situated at one side of the general nuclear substance, apparently extra-nuclear. This large karyosome next undergoes unequal division. The process takes place in a particular manner, which is neither amitotic nor yet a well-defined mitosis. This method of division has been usefully distinguished by Nägler (22) as "promitosis."

It may be as well to indicate, first of all, what is meant by promitosis. Its characteristic feature is that the division is initiated and carried out by means of an internal division-centre, which itself first divides, the two resulting daughter-centres then passing away from each other, but remaining connected by a distinct fibril or axial thread, the "centrodesmose." The term "promitosis" was originally applied by Nägler to nuclear division taking place in this manner, the intra-nuclear division-centre being a "nucleo-centrosome" or a karyosome. Where the karyosome plays this part, however, the true division-centre—certainly in most cases, and perhaps always—is an intra-karyosomatic centrosome or centriole, which initiates the process, although, owing to the intensity with which the karyosome usually stains, the centriole itself can rarely be distinguished, its presence being often only actually discernible at some other period in the development (cf. below, p. 182). Fortunately, however, the axial fibril or centrodesmose connecting the two separated daughter-centrioles persists often for a long time, even after the division of the karyosomatic or nuclear material is completed; hence it is just this stage of the division-process which is most likely to be observed. Therefore, where two nuclei (or karyosomes) are seen still connected by a definite centrodesmose, it may be safely concluded that the division has been brought about by an internal division-centre (centriole), in a promitotic manner. It only remains to say that I consider the term "promitotic division" can also be applied very suitably to the division of a karyosome, where this occurs unaccompanied by, or independently of, the division of the nuclens itself; Jollos (12) has already used the term in this connection.



It is undoubtedly in the above-described promitotic manner that the unequal division of the karyosome takes place in the young forms of *Karyolysus*; for I have found two or three examples which show very clearly the still persistent centrosome between the two halves (fig. 20). This fibril stretches apparently across the general nuclear mass; but it may really lie outside it, i. e. above or below; I do not feel sure upon the point. The smaller daughter-karyosome resulting from the division always comes to lie at the opposite side of the nucleus to the other, larger one (figs. 24, 26). This smaller, secondary karyosome, however, soon becomes incorporated with the general nuclear material; either it is distinguishable as a rather larger and more prominent grain, or else, probably having undergone further subdivision, it can be no longer distinguished from the rest of the chromatic substance. Now and again, it may be mentioned, in such a nucleus a small, but sharp and well-defined granule is seen in the centre; this may very likely be the centriole (fig. 40). The nucleus has by this time changed its position and passed to one end of the body of the parasite. In the majority, if not in most cases, it leaves behind it the larger half of the karyosome, which resulted, i. e., from the original promitotic division; this remains near the middle of the body, the nucleus simply moving away from it. Why this change in the nuclear position occurs I cannot say; it might be supposed, perhaps, that it had some connection with the commencing development of the U-form of the parasite, but the bending of the cytoplasm sometimes takes place at the end opposite to that to which the nucleus travels. Whatever the reason, this movement occurs, I should say, very rapidly, for I have not succeeded in finding an individual which shows the nucleus caught in the act, as it were, halfway between the end of the body and the stationary karyosome. This latter element thus left behind takes no further share in the nuclear development, and appears to be entirely discarded. As already indicated, it alters considerably in staining properties and in definiteness of outline; it gradually becomes

smaller and smaller, being perhaps partially used up by the cytoplasm, and ultimately its remains are seen at the surface of the body (figs. 34, 35). Often, however, no trace of this body is left (figs. 30, 38 and 39). On the other hand, occasionally this large karyosome seems to persist and to change its position with the nucleus (figs. 31, 37). In such cases it lies nearest to the end of the body, between this and the nucleus, having been pushed along as it were by the nucleus, instead of being left behind. Possibly the reason for this occasional persistence of the large karyosome as a separate element in close association with the nucleus, after the latter has changed its position, may be that the karyosome has not yet undergone the above-described division—a division which may be necessary in order to eliminate an unrequired portion of the karyosomatic material before the remainder is added to the nuclear substance. I have no evidence as to the further behaviour of the karyosome in these cases.

I can now summarise the general course of the early development in *Karyolysus*, so far as I was able to ascertain it. The different types of form observed are phases of one parasite. A small individual, such as that of fig. 21, penetrates a red blood-corpuscle (fig. 19) and begins to grow. As the parasite grows, changes in the nuclear constitution and position take place. At about the same time a definite envelope or capsule is formed around the parasite, inside which the latter tends to acquire, by bending up, a characteristic U-shape, and ultimately becomes stout and bean-like. The presence of the *Hæmogregarina* causes very great changes in the appearance of the host-cell, hypertrophy and pronounced alteration in the shape of the nucleus, sometimes its fission; further, the cytoplasm, or what remains of it, loses almost entirely its staining properties and becomes extremely difficult to see in the preparations.

From a comparison with Reichenow's valuable and detailed account (27) of the development of *Hæmogregarina stepanovi* of the tortoise, there can be little doubt that

the forms I have described of *Karyolysus* are phases in the development of the schizont, i. e. the form which undergoes schizogony or endogenous multiplication. A point in regard to which I cannot be certain is whether these young schizonts are the first to be developed, as the result of a fresh infection, or whether the infection is of some standing and these forms have been produced by a prior schizogony; in other words, whether the small, free individuals are developing sporozoites or merozoites. The only indication bearing upon the point which I can note is that the nuclear constitution of the young individuals, showing a distinct excentric karyosome, agrees markedly with the nuclear condition found in the developing merozoites of certain *Coccidia* and differs from that present in the sporozoites. I intend to discuss this agreement more particularly later, and will merely say here that this evidence favours the view that the schizonts which we have been considering are developing from merozoites.

#### The Question of the Specificity of the *Hæmogregarines* of Lizards.

I wish now to discuss the question of the specificity or true distinctness of certain of the many alleged species of *Hæmogregarine* (*Karyolysus*) which have been described from *Lacerta* spp., chiefly from the common European species *agilis*, *muralis* and *viridis*; my object is to show that some, at any rate, of these new species are almost certainly nothing more than different forms or phases of one and the same parasite, *Karyolysus lacertæ*. As I have had occasion to point out more than once in previous papers, the custom is far too prevalent of regarding any difference in appearance, or variation in size or form, observed in individuals of a certain genus of blood-parasites (and particularly in the case of *Trypanosomes* and *Hæmogregarines*), as indicating a distinct species, even though this "new species" occurs in a host in which a parasite of the same kind is already known. Often the view which is at least quite as probable, and in many instances more so, namely that the

forms in question are phases in the life-history of one and the same species of parasite, receives no consideration, and no attempt is made to connect the various types by means of intermediate stages. I am glad to see that Laveran and Pettit, in a recent note (15), also express a similar opinion, and comment upon the confusion liable to be caused by creating new species in the above casual manner.

To begin with the original description of *Hæmogregarines* from lizards, i. e. the account given by Danilewsky (6), this author observed various forms of the parasites in *L. agilis* and *viridis*. Making all allowance for the fact that Danilewsky's description and figures are mostly based on observations on the living parasites in the drawn blood,<sup>1</sup> and also for the primitive character of microscopical technique in those days, it seems probable nevertheless that this author was actually dealing with more than one species. Here, as in other cases (for instance, his memoirs on *Trypanosomes*), it is extremely difficult to gather what Danilewsky intended to mean by his grouping of different forms and the nomenclature he applied to them. He distinguishes three intra-cellular types (A, B and C), which he regards as having a genetic connection ("lien génétique") with one another. To these, collectively, he gives the name *Hæmogregarina lacertæ*; but immediately afterwards the second type (B) is termed *Drepanidium lacertarum*, because it is smaller and younger; while in another part of the memoir the third form (C) is called *Hæmocytozoon clavatum*! The last type is generally considered to be distinct; this is, I think, most likely, particularly since it does not produce, to judge from Danilewsky's account, hypertrophy of the blood-corpuscle and alteration of its nucleus; in other words, it is apparently

<sup>1</sup> While, of course, for many points, e. g. behaviour, movement, living observations are invaluable, it cannot be pretended that such can be relied upon where comparative questions of size, form and minute structure are concerned, especially in the case of intra-cellular blood-parasites, which, as is well known, frequently alter or else become deformed in drawn blood.

not a karyolysing form at all. The small type (v) may be a young phase of (A) ; more than this cannot be said. At any rate it is to the first described parasite (type  $\Delta$ ) that the specific name *lacertæ* really belongs. Comparing the different forms of the *Hæmogregarine* I have described above, from *L. muralis*, with Danilewsky's description and figures of *H. lacertæ*, it is perfectly clear that the parasite is the same species in both cases, and, moreover, in the same period of development ; some of Danilewsky's figures are of young forms, with the nucleus near the middle and the host-cell only slightly altered ; others are of the older phase, with the nucleus at one end and the nucleus of the corpuscle completely karyolysed.

The next account of *Hæmogregarines* from lizards was that of Labbé (13), who described parasites of this nature from *L. muralis*, *viridis* and *ocellata*. Labbé considered that the various forms which he observed belonged to two distinct genera, to which he gave the names *Karyolysus* and *Danilevskya* respectively. With the series of forms comprised in the latter genus we are not here concerned ; it is very doubtful whether any are included which should really be kept separate from the ordinary genus *Hæmogregarina*.<sup>1</sup> In the genus *Karyolysus*

<sup>1</sup> It may be noted, however, that Labbé seems to have paid no regard at all to the laws and standards of nomenclature, for he deliberately placed in this genus the parasite of *Cistudo europæa*, originally described by Danilewsky under the name *Hæmogregarina stepanovi*, that is to say, the type-species of the genus *Hæmogregarina* in other words, at his own pleasure, he replaced the generic name *Hæmogregarina* by that of *Danilevskya*. If he wished thus to commemorate the Russian savant's name he ought, of course, to have called the parasite which he distinguished as *Karyolysus* by his name instead. Moreover, for the species of "*Danilevskya*" which he found in lizards he created the name *lacazei*, although saying at the time that this was probably the same form as that distinguished by Danilewsky as *Hæmocytozoon clavatum*. In any case, therefore, this *Hæmogregarine* of lizards should bear the specific name *clavatum* (not *lacazei*), and if it does not belong to the genus *Hæmogregarina*, the generic name *Hæmocytozoon*, not *Danilevskya*, must be given to it.

Labbé placed forms which he regarded as similar to those described first by Danilewsky under the designation *H. lacertæ*. Why, in so doing, he altered the specific name to *lacertarum* it is difficult to understand; the name should read, of course, *K. lacertæ* (Danil.). From a study of Labbé's description I do not think there is any reason to doubt that this author was dealing, in the main, with Danilewsky's parasite, *H. lacertæ*; though it is true that certain of his figures may represent some other *Hæmogregarine*. Unfortunately, Labbé does not give any details about the particular species of lizard in which the various types of the parasite he figures respectively occurred. Since he examined four different species of host, in certain of which, at any rate, another *Hæmogregarine* is also parasitic (as, indeed, he recognised, distinguishing this latter by the name "*Danilevskya*" *lacazei*, see footnote, p. 186), it is quite possible that he did not altogether succeed in separating the two forms. Nevertheless, leaving out of consideration his description of the "endoglobular sporulation,"<sup>1</sup> Labbé's account of the appearance, size and structure of the young and adult parasites in the blood-corpuscles, and in particular his description of the marked alterations in the host-cell, make it perfectly evident that most of his observations did actually refer to the same parasite as that described by Danilewsky, and as that which I found in the lizards I examined.

In 1901, Marceau gave an account (18) of the *Hæmogregarine* parasites which he observed in *L. muralis*, and in this lizard alone; and here also it is quite obvious that the author was dealing chiefly, if not entirely, with *K. lacertæ*. On the whole, Marceau's description agrees closely with that of Labbé.

It is sufficiently clear, I think, that there is a definite

<sup>1</sup> This process doubtless represents the schizogony of the parasite, which is apparently either of a double character, similar to that described by Reichenow (27) in the case of *H. stepanovi*, or else of a type where sexual differentiation is already manifest.

parasite, occurring in *L. muralis* and probably also in *L. agilis* and *viridis*, for which the specific name *lacertæ* must be retained. Further, in my opinion, it is also preferable to retain Labbé's distinct generic name *Karyolysus* for this *Hæmogregarina*, as also for any other similar form which may produce the same characteristic effects upon the host-cell; I certainly consider such forms can be advantageously grouped together—if not in a separate genus, at any rate in a distinct sub-genus—on account of their peculiar behaviour in this respect. It is only necessary to compare the effect on its host-cell produced by an ordinary *Hæmogregarina* to realise that there is a marked difference between the two types of parasite. Species of the genus *Hæmogregarina*, whether from fishes or reptiles, may often cause more or less hypertrophy of the red blood-corpuscle; but they never stimulate, as it were, the cell-nucleus to undergo such profound changes as is the case with *Karyolysus*, where the nuclear alteration begins, as I have shown above, almost as soon as the parasite has invaded the corpuscle. I need only refer, by way of illustration, to the recent figures published by Minchin and myself (*loc. cit.*) of *H. triglæ*, by Neumann (23) of various piscine *Hæmogregarines*, by Reichenow (*loc. cit.*) and also Hahn (9) of *H. stepanovi*, and lastly, the figures of many species from snakes given by Sambon and Seligmann (29)<sup>1</sup>. In all these cases the host-cell nucleus is practically unaltered; it may be now and then slightly flattened in appearance, but this is usually where it has been pushed to one side of the cell by the growing parasite, and is obviously due to a mechanical cause. It may be said, of course, that if a separate genus *Karyolysus* is to be thus recognised, the distinction between it and *Hæmogregarina* will be based mainly, if not entirely, on biological grounds. This is, no doubt, true; but one has not to look far for other instances where a generic distinction, which is generally accepted, is recognised for biological

<sup>1</sup> Some of these last should clearly be placed in the genus *Karyolysus*.



reasons, which, if not just the same, are of a similar order. Thus the avian blood-parasite known as *Leucocytozoon* is distinguished from that known as *Halteridium*, although there is little doubt that the two types are very similar in structure and in regard to the essential features of the life-history; the principle difference is that of habitat, the one form (*Halteridium*) being parasitic in the red corpuscles, the other (*Leucocytozoon*) in the uninuclear leucocytes.<sup>1</sup> Nevertheless, it is very useful to continue to distinguish the two types as separate genera. And similarly as regards these reptilian blood-parasites, as a means of indicating at once the characteristic difference in the effects on the host-cell, it is most convenient to retain the generic names *Karyolysus* and *Hæmogregarina* for the karyolysing and non-karyolysing group of species respectively.

Of late years several workers have given accounts of *Hæmogregarines* from lizards, for the most part recording the occurrence of new parasites—or at any rate, parasites regarded as new—in various additional hosts; several of these are undoubtedly *Karyolysus*-forms. The parasites of the different species of *Lacerta* have been studied chiefly by Laveran and Pettit and by França. In their first paper, Laveran and Pettit (14) describe the parasites they observed in *L. muralis* and *viridis*, more frequently in the former species. They distinguish three different types, all of which they consider to represent Danilewsky's parasite, which they term *H. lacertæ*; the authors thus use the correct specific name, but prefer to keep the parasite in the genus *Hæmogregarina*. The first two types are the same as those which I have again found, that is to say, young schizonts and older ones. The only point which requires notice is that the authors consider there is no capsule, but merely a shrinkage space around the second type of form; this is certainly a mistaken view on their part. The third form of parasite is, in my

<sup>1</sup> The different habitat explains, of course, the fact that the one parasite (*Halteridium*) produces melanin pigment, while the other (*Leucocytozoon*) does not.

opinion, a type rather different from any phase so far described by other workers, and from anything I have observed. It is a large, curved form, certainly a Karyolysus, because of its effect on the cell-nucleus; I should say it probably represents another phase of *K. lacertæ*, but until the life-cycle is better known or until this form has been connected by intermediate stages with other known phases, the matter must remain uncertain.

França, in a series of papers on the *Hæmogregarines* of lizards, chiefly species of *Lacerta*, has been unfortunately preoccupied with the idea that almost every variety in form and appearance of parasite observed represents a distinct and independent type, with the result that he has greatly complicated and confused the subject of these *Hæmogregarines* of lizards. Thus, in more than one case, the author creates several new species for parasites from the same host, in some instances basing the distinctions between them on such slender grounds as the different staining appearances (tint of colour, presence or absence of granulations, etc.) exhibited. Now, França's figures are all from preparations stained by some modification of the Romanowsky method; and, as is well known, the great variability and uncertainty in the staining appearance presented often by the same object at different times, even where the smear has been treated, so far as was known, in exactly the same manner, renders it perfectly useless to label as distinct species forms showing differences in appearance after being stained by a Romanowsky method, mainly or solely on this ground. Again, França is of the opinion that it is unlikely that a particular species of host will be infected with the same species of parasite in different countries, or even in different districts of the same country. I can only say I do not share this view at all. We know, for example, that *Trypanosoma lewisi* occurs in rats all over the world; and other common parasites, e. g. certain *Gregarines* and *Coccidia*, are known from the same species of host in various countries. I do not think there is any reason to doubt that the same species of *Hæmogregarine* may occur in the L.

muralis of Portugal, for example, which is found in that lizard in Russia, and again in Southern Austria, and in France. I associate myself entirely with the remarks of Laveran and Pettit in their later note (15) with regard to this matter.

In one of his memoirs (8), França describes the different forms of hæmogregarine which he found in *L. muralis* in Portugal. The author leaves out of account altogether the species *K. (H.) lacertæ*. This he does for two reasons: firstly, in accordance with the view just referred to, because of the different geographical locality of the host in the case of the lizards which he examined; and secondly on the ground that several different forms have been really included in the specific designation *lacertæ*. From what I have shown above, it will be evident that, on the contrary, we can recognise and clearly distinguish a well-defined species, to which the name *lacertæ* belongs by right.

França creates no fewer than four new species, all from this one host, namely, *H. nobrei*, *bicapsulata*, *marceani* and *nana*. These different parasites usually occur associated together in various groupings; and it is the exception rather than the rule to find them separately. The first three are typical karyolysing forms, and hence may be termed *Karyolysus*. The last named, it should be pointed out, is, as its name implies, a very small form. From the only figure given it is obvious that this is merely a young phase; it cannot itself be regarded as an adult parasite, and in its older phases it may possibly be identical with one of the other types described. At any rate, it seems distinctly premature, in the circumstances, to give this type a new specific name. As regards França's other three species, I confess straightway that I consider they are only different forms or phases of our old friend *K. lacertæ*. I have come to this conclusion principally on two grounds; in the first place as a result of the detailed comparison I have myself made of certain forms of *K. lacertæ*, and of the alterations produced in the infected host-cells as seen in smears stained with Giemsa and also in wet preparations stained with iron-hæmatoxylin; and secondly,

as a result of the valuable light thrown on the whole subject of the life-cycle of a *Hæmogregarine* by Reichenow's work on *H. stepanovi*. Of course, this work has appeared since França's papers were published, so that we have now a guide to the interpretation of the various phases which was then unavailable. As the general scheme of the life-cycle, so far as it is undergone in the Vertebrate host, has been shown by Miss Robertson (28) to be fundamentally similar in the case of another *Hæmogregarine* also, I think we may regard it as probable that the life-cycle is similar, in its main traits, in other reptilian *Hæmogregarines*; and there is no need to consider that of *Karyolysus* as likely to be very different from that of *Hæmogregarina* merely because of the biological differences between the two forms, i.e. with respect to the behaviour and reaction of the host-cells. Assuming a general agreement, a particular type or stage of parasite observed in a lizard might represent any of the following phases in the life-cycle of a single species: The young growing schizonts produced from the sporozoites in a new infection; the merozoites or growing schizonts resulting from a first type of schizogony, e.g. with many merozoites (micromerozoites?); the merozoites or young schizonts resulting from a second type of schizogony, e.g. with few merozoites (macromerozoites?); lastly, the growing gametocytes, which may themselves be differentiated. As these various phases very likely show definite, though it may be slight distinctions from one another, if they were only observed casually, as it were, and their further development was not followed, nor their connection with one another ascertained, some would at once jump to the erroneous conclusion that they constituted distinct and new species.

Considering França's three *Karyolysus*-forms separately, *K. (H.) bicapsulata*, which we may take first, is so named because of two caps of deeply staining matter which occur one at each end of the parasite. From França's fig. 7 it is seen very clearly, in the first place, that these "caps" are distinctly outside the true envelope or capsule of the *Hæmogregarine*, and secondly, that they resemble closely in appear-

ance the hypertrophied nucleus of the host-cell, and, in fact, may be connected with the latter (for instance, the cap on the right-hand side of the upper parasite of fig. 7). Now, as stated above, I have observed a very similar appearance in some individuals of *K. lacertæ* in Giemsa-stained smears (cf. figs. 16-18). In my opinion there is no doubt whatever that these "caps," in the case of França's parasite also, are simply the result of the alteration to the nucleus, the thicker or club-shaped end-parts of which curve round the parasite and may be almost or quite detached from the middle portion; these caps have nothing whatever to do, directly, with the parasite. A perfectly similar behaviour of the nucleus of the blood-corpuscle has been described by Billet [2] in the case of *K. (H.) curvirostris*; two of this author's figures show exactly the same condition. Other points about França's account of *K. "bicapsulata,"* e.g. the average size, the presence of a definite envelope around the parasite, make me practically certain in my own mind that this is not a new species at all, but only a phase of *K. lacertæ* corresponding to the second, older type described above. I should add, however, that Laveran and Pettit also seem to regard this "*bicapsulata*" as a distinct species, although they say that they found it associated with *lacertæ*, and mention further that, in deeply stained specimens, the "caps" stain very similarly to the deformed host-cell nucleus!

*K. (H.) nobrei.* This form Laveran and Pettit (*loc. cit.*) themselves consider resembles *K. lacertæ* so closely that it is doubtful whether it is really a distinct species. In my own preparations I have not come across any individuals which exactly represent this form; the parasite drawn in fig. 15, however, shows considerable resemblance in size and general appearance to the form figured by França in his fig. 2, the chief difference being in the position of the nucleus, which is near the middle of the parasite in França's case. I should say it is very likely that this is just one of those cases referred to above, where a different phase in the life-cycle of the parasite has come under observation. From a consideration

of França's figures relating to *K. nobrei*, the suggestion may perhaps be put forward that the phase in question corresponds to the second process of schizogony (with few merozoites) which occurs in *H. stepanovi*, and the type of individual immediately preceding or resulting from the same.

Again, with regard to *K. marceani*, a form occurring in the blood is practically indistinguishable, according to certain of França's figures, from some individuals of the second type of *K. lacertæ*, which I have described; thus my figs. 9 and 12 agree closely with his figs. 9 and 10 respectively of *K. marceani*. França also mentions and figures certain phases from the liver, which he considers represent conjugation. What these do exactly signify is uncertain, but the micro- and macrosporogony described as resulting from this process is quite comparable to Marcean's account of the same process in what is admittedly *K. lacertæ*. (It may be added that in both cases it is of course much more probable, considering the matter in the light of Reichenow's work, that schizogonic multiplication is concerned.) Hence, on the whole, and at any rate until the life-cycle of *K. lacertæ* has been thoroughly worked out, it is very much better, I think, not to adopt these new names, *bicapsulata*, *nobrei* and *marceani*, which would only entail great confusion and difficulty, but to consider them as representing merely different phases of *K. lacertæ*.

To complete my summary of this question, I must mention that there has been the same premature and probably useless multiplication of species in the case of *Karyolysus*-forms from another species of *Lacerta*, viz. *L. ocellata*. In the first place, Billet [2] gave a short account, already referred to, of a karyolysing *Hæmogregarine* occurring in this lizard in Algeria, to which he gave the specific name *curvirostris*. As this parasite occurs in a different species of host, we may perhaps assume for the present that it is a form distinct from *K. lacertæ*, though I do not think this can be regarded as at all certain. A few weeks later, Nicolle [24] also described a similar *Hæmogregarine*, from a variety

of *L. ocellata* in Tunis, which he considered to be distinct from *curvirostris* and called *biretorta*. Lastly, França [7], not content with these two, makes three additional species, *H. [K.] schaudinni*, *nicollei* and *minuta*, to say nothing about a variety *africana* of his first one, all from *L. ocellata*. Thus in two species of *Lacerta*, namely *muralis* and *ocellata*, there are according to França no less than ten species of *Hæmogregarine*. Is not this carrying species-splitting to an absurd degree?

I have not studied the parasites of *L. ocellata* myself, but having regard to the above analysis of the so-called species of *L. muralis*, some of those from this other lizard must be viewed with great suspicion. For instance, *biretorta* is almost certainly the same parasite as *curvirostris*, and hence a synonym of the latter; this is clear to my mind, from França's figs. 15 and 17 (*loc. cit.*), and, indeed, Laveran and Pettit, in a note I have not been able to see,<sup>1</sup> have also thrown doubt upon the independent nature of *biretorta*. The same conclusion applies to França's species *nicollei*, which the author himself admits has considerable resemblance to *curvirostris* and *biretorta*; in short from França's fig. 18 it is obviously only a slightly different phase of *K. curvirostris*. The parasite termed by França *schaudinni* appears rather different in character both from *lacertæ* and *curvirostris*, although França's fig. 2 of this form is remarkably like my fig. 4 of *K. lacertæ*; it may perhaps be left an open question whether *schaudinni* is some other phase in the developmental cycle of *K. curvirostris* or a distinct species. It is rather odd, however, that França has included as a particular form of *curvirostris* a type (*vide* his fig. 16) which is undoubtedly only a form of his *schaudinni*! I conclude the subject by registering a strong protest against this habit of creating a new species on entirely insufficient grounds.

<sup>1</sup> Bull. Soc. Path. exot., ii, 1909, p. 377.



### III. COMPARISON OF THE NUCLEAR CONDITION IN KARYOLYSIS LACERTÆ AND CERTAIN OTHER HÆMOGREGARINES WITH THAT OF COCCIDIA; THE QUESTION OF THE KARYOSOME AND THE INTRA-NUCLEAR DIVISION-CENTRE.

I propose next to compare the nuclear condition, as I have described it above in *Karyolysns lacertæ*, with that which is found in certain *Coccidia*, at a particular period in the life-cycle, since, in my opinion, the agreement shown affords an important indication of the close affinity and phylogenetic relationship of these two types of parasite. This resemblance is especially marked in the case of the merozoites and very young schizonts of a *Coccidian*, which is, according to Shellack and Reichenow (32) really *Barrouxia alpina*, Léger; this phase, it must be mentioned, has for long been mistakenly included in the life-cycle of *Adelea ovata*, of which it was considered to represent the male type of schizogony. The structural details of this particular stage or form of the parasite were first described by Siedlecki (33), and further notes with regard to it have since been given by, among others, Jollos (12), both these authors having included it in the cycle of *Adelea*.<sup>1</sup> In order to facilitate the comparison with the *Hæmogregarine*, I have drawn (Pl. 9, figs. 41-43; Pl. 10, figs. 1-3) some individual merozoites from an original preparation of my own, these parasites being easily obtainable in centipedes. Although I have found exactly the same nuclear condition and behaviour in this early phase which has been observed by Jollos, I think it is worth while to describe it again, because doubts have been recently cast upon Jollos' account, both as regards these points and others.

At first the young schizont of *Barrouxia*, which may be

<sup>1</sup> The two forms are parasitic in the same host, *Lithobius forficatus*; this fact is, of course, chiefly responsible for their different phases having been confused together.

little more than a merozoite,<sup>1</sup> has a single large karyosome placed quite at one side of the general nuclear substance; the latter is finely granular in character, and does not stain deeply, the granules being fairly uniform in size and appearance. More frequently a rather later condition or phase is found, in which there are two karyosomes, generally at opposite sides of the principal nuclear mass; these two karyosomes are usually more or less unequal in size, and neither is so large as when there is only one. I have been much exercised in regard to the question of the true situation of these karyosomes. In nearly all the individuals a well-marked clear zone, which in some cases is relatively wide, surrounds both the general nuclear substance and the karyosomes (or karyosome). Is this clear zone to be considered merely as a shrinking-space, separating the whole of the nuclear organellæ from the general cytoplasm of the parasite, or is this area really within, and therefore a part of, the nucleus, the limit or border of which is on the outer side of the clear area and in contact with the edge or margin of the surrounding cytoplasm? In the former case, of course, the karyosomes would be actually extra-nuclear; in the latter they would be within the nucleus, but excentrically placed, near the periphery. After some hesitation I have come to the conclusion that the latter view is the correct one, and that the pale, clear area really constitutes the peripheral region of the nucleus. In the case of most individuals I have found it almost impossible to satisfy myself of the existence of a definite membrane, bordering this zone externally, as distinct from the edge or margin of the surrounding cytoplasm itself; and the same difficulty has presented itself apparently to other observers, if one may judge from certain of their figures (e.g. Siedlecki's fig. 17 and Jollos' figs. 22 and 28). Moreover, the limit of the centrally situated, uniformly granular,

<sup>1</sup> The earliest change in the condition of the karyosome, namely its division into two, may even take place before the fully formed merozoite has been liberated from the "barillet" of which it has constituted a segment.

nuclear material is at times so sharp and well-defined that it might almost be regarded as a membrane. However, now and again one is fortunate enough to be able to focus a definite line bordering the pale area in question on the outside, which most probably represents a true nuclear membrane. And there are one or two other reasons which support this view. Thus Siedlecki (*loc. cit.*) states that he observed this clear zone in these forms of the parasite even in the living condition, which shows that, in the strained preparations, it cannot represent merely a shrinkage-space. Further, although this zone appears so clear and pale by comparison with the parts of the parasite immediately surrounding, it is, nevertheless, occupied by something—probably in the nature of nuclear sap—which is extremely faintly stained; that this is actually the case is sometimes shown distinctly because of a peculiar condition or appearance which is often, but not always, presented by the karyosomes. These elements themselves, especially the larger ones, *i.e.* when there are only one or two, may be surrounded by a perfectly clear halo-like circle, which is quite colourless; this halo round the karyosome passes between it and the central nuclear substance, indenting the surface of the latter, so that it forms a concavity or cup as it were. The difference between this small, quite colourless zone and the almost clear, faintly staining area, extending around the periphery of the whole nucleus is sufficiently conspicuous. To sum up the matter, therefore, the karyosomes must be considered as really intra-nuclear, situated in a peripheral zone, which is very pale, and apparently consists only of nuclear sap, the rest of the nuclear material, containing a small amount of chromatin being aggregated to form a central mass. I have not been able to see any delicate threads or rays passing from this central mass to the limiting membrane of the nucleus, and traversing the faintly-stained, peripheral zone, nor does Jollos (*loc. cit.*) mention or figure anything of the kind; but Chagas (5) has described and figured “linin threads,” having such a disposition in the case of somewhat older phases of a new species of

Coccidian ("Adelea" hartmanni),<sup>1</sup> in which the nuclear constitution and behaviour of the young schizont is very similar (cf. also below, Note IV, where the nucleus of Leucocytozoon is compared).

To return now to the behaviour of the karyosome. The two subequal or unequal karyosomes result undoubtedly from the division of the original large, single karyosome, which takes place in a promitotic manner; for in a couple of instances I have found the centrodesmose still persisting (cf. fig. 42). There is no possible doubt about this division of the karyosome; the process here appears to be just the same as in *Karyolysus lacertæ*, and my having found it in both parasites substantiates and corroborates Jollos' account of this behaviour of the karyosome in the young schizonts of this Coccidian. While the early condition and behaviour of the karyosome during this period is thus completely paralleled by the above-described early phase of *K. lacertæ*, the subsequent course of events differs slightly in the two parasites. In the Coccidian, at a rather late stage, three or four karyosomes are present (fig. 43, also fig. 3, Pl. 10), most of which are small and have obviously arisen by the further division of one or both of the two above-mentioned daughter-karyosomes (cf. also Jollos' figure).<sup>2</sup> That is to say, here the karyosome continues to be separate and distinct from the general nuclear substance (as is known from the ascertained further development), whereas in *K. lacertæ* the karyosomatic chromatin which is retained by the nucleus becomes distributed amongst the general chromatic substance and no longer distinguishable.

It is necessary to emphasise this fact of the promitotic division of the karyosome because, in recent papers, Reichenow

<sup>1</sup> This parasite is regarded by Léger (16) as the type of a new genus, *Chagasia*.

<sup>2</sup> It may be recalled that Siedlecki himself, in his original description of this form, also states that the karyosome divides: thus, "il [le karyosome] donne, par bourgeonnement, naissance à des karyosomes secondaires," and, again, "surtout un karyosome, parfois divisé en deux ou trois fragments."

(27) and Schellack and Reichenow (32) have maintained that no division of the karyosome occurs in the above phase of *Barrouxia* ("Adelea"), and consider that the secondary karyosomes (i. e. the daughter-karyosomes) arise *de novo*, by independent formation from the general nuclear substance; in regard to this detail the authors are certainly mistaken. Moreover, quite recently Chagas (*loc. cit.*) describes and figures, in his account of *Chagasia* (*Adelea*) *hartmanni*, perfectly similar promitotic divisions of the karyosome in different phases of the life-history. I have a strong idea that Reichenow and Schellack, in arriving at the above conclusion, have been influenced—if unconsciously—by the view which one of them (Reichenow) seems to have formed upon the question of the karyosome, its nature and significance, as a result of his work on *Hæmogregarina stepanovi*. No one is more sensible than am I of the great value of Reichenow's research, which has thrown full light upon the complicated subject of the *Hæmogregarine* life-cycle; but in regard to this somewhat important cytological question I find myself obliged to differ from him.

Hartmann and Chagas (10) have suggested that the reason for this may be that as the particular parasite (*Hæmogregarina stepanovi*) upon which Reichenow worked is a very small one, the observation of minute cytological details and changes would be rendered more difficult and hence they may have escaped detection. I do not altogether share this opinion; for one thing, I do not think *H. stepanovi* is much, if any, smaller than the small forms of *K. lacertæ*, where the karyosome and its division can be made out without difficulty. I am inclined to consider that, on the whole, the nuclear constitution and behaviour in *H. stepanovi* is as Reichenow has described it; and therefore, as a logical sequel, that this species of *Hæmogregarine* differs in one or two cytological respects, such as the absence of a typical karyosome, from certain other *Hæmogregarines* and certain *Coccidia*. This is the more probable, in my opinion, because of a fact which is evident on scrutinising Reichenow's figures, namely, that the

chromatin of the general nuclear substance is very much more prominent, i. e. in the form of numerous fairly large, deeply staining grains, than is often the case in the corresponding phases of other parasites where a karyosome is present; and just the same condition is seen in the closely allied species, *H. nicoriae*, according to Miss Robertson's description (loc. cit.). If the nuclear appearance of these parasites is compared with that, for instance, of the young phases of either *K. lacertae*, *H. gracilis* (Wenyon [36]), *H. lutzi* (Hartmann and Chagas [10]), or of *Barrouxia alpina* ("Adelea ovata") or *Chagasia hartmanni*, a striking difference is at once apparent; in the latter, most, sometimes nearly all, of the chromatin is contained, for the time being, in a distinct karyosome (or more than one). It is especially in regard to this absence of a definite karyosome that the two species of *Hæmogregarine* from tortoises are interesting. Thus, Miss Robertson expressly states that "at no stage does *H. nicoriae* show in its nucleus the karyosome so characteristic of *Coccidia*." Now, in my opinion, *H. stepanovi* shows an important intermediate condition between the type of nucleus possessing a karyosome, as in the above examples, and a type like that of *H. nicoriae*, where this organella is quite wanting. According to Reichenow, *H. stepanovi* has at certain periods of its life-cycle (which, in general, correspond to the phases when a karyosome is present in other forms) a definite rounded body, situated near the periphery of the nucleus, which is always very pale and faintly stained and appears quite different from the prominent chromatic grains.

Reichenow uses the term "nucleolus" for this body, and this is most probably the correct name for this particular structure, and indicates its true nature; but my reason for thinking so is not exactly the same as that given by Reichenow. It seems clear from the author's description and figures that the body in question contains little or no chromatin; it corresponds apparently to the true nucleolus of an ordinary tissue-cell, i. e. a body consisting simply of

plastin or allied material. Reichenow, however, regards this element as a nucleolus principally on the ground of its behaviour during nuclear division, that is to say, its disappearance and re-formation at different periods. Unfortunately, Reichenow's observations on this body in *H. stepanovi*, which have led him to the conclusion that it has the physiological significance of an ordinary nucleolus, have prejudiced his view upon the true karyosome, which is something quite different. He has, in my opinion, failed to grasp what is the really essential feature of a true karyosome, namely, that it is a chromatin-nucleolus, an organelle which holds or contains a large proportion of the entire chromatic substance of the nucleus. His only reference to this fundamentally important character is seen in the following sentence:—"Was ihn [d. h. den Binnenkörper (Karyosom)] von dem echten Nucleolus unterscheidet ist, abgesehen von seinem Chromatingehalt auf den wir keinen grossen Wert legen dürfen, allein der Umstand, dass er bei der Kernteilung erhalten bleibt" (the spacing is mine). Because he thus ascribes no importance to this, the principal feature of the karyosome, he is able to persuade himself that the typical "Binnenkörper" or karyosome in other cases is the equivalent, practically speaking, of the body he has described in *H. stepanovi*.

Further, Reichenow brushes aside as quite untenable the usually accepted view that the karyosome behaves as an intra-nuclear division-centre, which is founded on the reliable observations of many previous workers. The admitted existence of the "Hantel-Figur" he endeavours to explain by supposing that it is produced by the karyosome being drawn out into two parts by the separating halves of the dividing nucleus. He appears to have adopted this attitude on two grounds: in the first place, because he has found that the nucleolus of *H. stepanovi* does not so divide, and secondly, because he evidently doubts the existence at all of an intra-karyosomic centrosome and the occurrence of promitotic division, so far as the *Coccidia* and *Hæmosporidia* are con-



cerned. In regard to the first point, the very fact that the organella seen in *H. stepanovi* is a nucleolus and not a karyosome explains why it does not divide, as I hope to show below (cf. pp. 213 and 214).

With regard to Reichenow's doubts about the occurrence of promitotic division and the presence of an intra-karyosomatic centrosome, I must say I think they are quite unfounded. In the first place, both my own observations on the same Coccidian and those of Chagas on an allied form support Jollos' account (*loc. cit.*) in so far as regards this detail. Further, I have found a precisely similar division of the karyosome by means of a centrodesmose in an early phase of the *Hæmogregarine*, *Karyolysus lacertæ*.<sup>1</sup> And, as I have previously remarked, the presence of a centriole within the karyosome may be legitimately and reasonably assumed where the occurrence of a centrodesmose is noted. From a study of Trypanosomes, I know how difficult it often is to actually distinguish the centrosome, even in the large karyosome of a relatively large individual, although the occurrence of a centrodesmose in the division of the karyosome (e. g. of the trophonucleus) has long been well known. Nevertheless, Minchin and I, in our notes on *T. raia* (20), clearly demonstrated the actual presence of a centrosome in the resting karyosome. Moreover, as regards the *Hæmogregarines*, since Reichenow's paper appeared, some interesting observations on the leucocytic parasite of the dog, *Hepatozoon* (*Hæmogregarina*) *canis*, have been published by Wenyon (37). Here, too, a distinct promitotic division of the karyosome is figured; and in the case of this parasite, the karyosome is relatively very small in some phases, when it probably represents little more than the centrosome itself. Even in the nucleus of *H. stepanovi*, it is not impossible that a centriole is really also present, and it is just in regard to this detail that I think the suggestion of Hartmann and Chagas (10) may apply, namely, that this minute granule may have escaped recognition owing to the difficulty of distinguishing it amid the

<sup>1</sup> Cf. also footnote to p. 205.

more prominent chromatic grains. In this connection it must be noted that Miss Robertson (28) mentions and frequently figures a small but definite granule in the nucleus of *H. nicoriæ*, which is in no way distinguishable from the peripheral chromatin grains in size or staining reaction, but which nevertheless appears to be different from the other nuclear elements in so far that, in the primitive type of nuclear division, it seems to form a centrodesmose. This minute body may well be the centrosome; just as the central granule which I have sometimes noted in the nucleus of *K. lacertæ*, when there is no longer a distinct karyosome, is also probably one (cf. fig. 40).

It is a pity that Reichenow, in his able memoir, should have thought himself at liberty to disregard or treat as negligible the evidence afforded by the research of other earlier workers, such as the classic instances of *Coccidium schubergi* and *Cyclospora caryolytica*, made known by Schaudinn (30 and 31), which pointed clearly to the existence of this characteristic promitotic division of the karyosome in the respective parasites, and which has since been abundantly corroborated in other cases; to say nothing of his having entirely failed to take into consideration that in several of the lower Flagellates the occurrence of a centrosome and of promitotic division of the karyosome is now well established. As it is generally agreed to-day that the Ectospora (Telosporidia) are descended from Flagellate ancestors, it might be expected, on *à priori* grounds alone, that among Coccidia and Hæmosporidia some would be found to exhibit a similar mechanism in their nuclear division.

I certainly do not think it is advisable to adopt such a comprehensive generalisation as that postulated by Hartmann and Chagas and the followers of their school, namely, that a central organella (centrosome) is present, as a general rule, in the karyosome of all Protozoa; but I will at once admit that I consider this idea considerably nearer the truth than the view maintained by Reichenow, that a centrosome is not present in the karyosome in any of the cases mentioned

above, and that no promitotic division of this body occurs.<sup>1</sup>

### Nature and Significance of the Karyosome.

I have laid stress upon this fact of the presence of an intrakaryosomatic centrosome because of the important bearing it has upon the question of the real nature and significance of the karyosome, and because it helps to explain satisfactorily the different behaviour of this body in different phases of the life-history. In the first place, it is necessary to clear the ground of what I consider is a serious misconception of the karyosome, which is largely fostered by the school of Hartmann, Nägler and others, and which appears to be based upon the fact that this organella frequently leads the way in nuclear division, and contains within itself a division-centre. Now, the primary and principal meaning of the term karyosome is chromatin-nucleolus, i. e. a body consisting of a plastin basis impregnated with chromatin; it might be considered unnecessary at the present day to have to emphasise this essential character, but that this is not so is shown by Reichenow's reference to it as one "auf den wir keinen grossen Wert legen dürfen!" This is the sense in which the word was first used, and on account of which it has been adopted by most authors (cf. Labbé (13), Minchin (19), Siedlecki (33 and 34), Wilson (38) and others). Schandinn, in his celebrated memoir on the *Coccidia* of *Lithobius* also says: "Jeden Falls unterscheidet sich das Karyosom der Coccidien von den echten Nucleolen der Metazoenzellen scharf durch seinen Chromatingehalt." But in many recent papers by members of the school of thought referred to above, a strong tendency is noticeable to assume that the possession of a centrosome and of the function of acting as a division-centre is to be definitely associated with the idea of a karyosome as a whole and to be implied in the meaning of the

<sup>1</sup> See also the account given in Note IV of the nuclear structure of *Leucocytozoon* and *Halteridium*, in both of which division-centre and centrodosome are clearly shown.

term, as a definite attribute of this body; thus, Hartmann and Chagas (11) say: "Man kann daraufhin jetzt den Begriff des Karyosoms direkt von dem Vorhandensein eines Centrioles [Centrosoms] abhängig machen." This notion has been elaborated to such an extent that the whole karyosome, that is to say, chromatin-nucleolus + centrosome, has come to be regarded as a distinct entity, a locomotor or kinetic centre; its chromatin is the "kinetic component," while the surrounding chromatin, scattered through the nucleoplasm or nuclear sap, is the "generative component" (the second nuclear type of Hartmann and Chagas).

Now, in my opinion, this idea of the karyosome is very forced, besides being really quite unsupported by any evidence. For one thing, I do not consider that the whole karyosome (i.e. chromatin-nucleolus + centrosome) can be regarded as representing a definite unit or "locomotor-centre"; it may happen, in fact, that the intrinsic division-centre is outside and distinct from the karyosome (as in *Spongomonas*, for example, figured by Hartmann and Chagas, and cf. also the "nucleo-centrosome" of *Adelea zonula*, according to Moroff (21). Again, the condition shown by the true Binucleata, the Trypanosomes and their allies is quite against this interpretation. Here there are two separate nuclei—a locomotor nucleus (kinetonucleus) and a vegetative one (trophonucleus); to this, of course, Hartmann and Chagas assent, saying (*loc. cit.*) that "zwei verschieden differenzierte Kerne in der Zelle vorhanden sind, einer [trophonucleus] vorwiegend mit der trophisch-generativen Komponente, der andere [kinetonucleus] vorwiegend mit der lokomotorischen Komponente." But nothing is more certain than that the trophonucleus of a Trypanosome possesses a large, conspicuous karyosome, containing most of the chromatin of the nucleus, and also a distinct centrosome (centriole)! If, therefore, the karyosome in this case is a trophic component (which is, indeed, the most reasonable view to take), whatever ground is there for supposing that, in the passive, intra-cellular Coccidian, the equally large and

conspicuous karyosome represents a kinetic (locomotor) component? Moreover, another idea prevalent in the writings of the adherents of this school, which is strongly to be deprecated, is that of contrasting, as two opposed constituents, kinetic and generative components of the nucleus. These two things are not strictly comparable or opposable at all. On the one hand, the essential kinetic components are the achromatic elements—centrosome, centrodesmose, and so on; and in all probability these take part in effecting the division of generative chromatin as well as of vegetative (trophic) chromatin. And, on the other hand, where a separate kinetonucleus is present, which may be regarded as standing in a special relation to the locomotor activities of the Trypanosome, there is no reason whatever for supposing that the chromatin of this nucleus is less generative in character than that of the trophonucleus.<sup>1</sup> In short, I cannot share the above view of the locomotor or kinetic nature of the karyosome as a whole at all; it is the contained centrosome, not the chromatin-nucleolus, that brings about the division. The so-called "Hantel-Figur" is really the result of the gradual (passive) separation of the two halves of the karyosome as the centrodesmose extends.<sup>2</sup>

It seems to me very much better to return to the earlier manner of regarding the karyosome, which has been well set forth and discussed by Siedlecki (34 and 35), namely, that it is an organella, whose principal function is to store up reserve chromatin—and particularly trophic as distinct from generative chromatin—for use as and when required by the nucleus, or, as the case may be, for elimination if not required. This theory undoubtedly fits in best with the known variations in

<sup>1</sup> This point was emphasised by me so long ago as 1906 in my analysis (40) of Schaudinn's celebrated work on the parasites of the little owl.

<sup>2</sup> The same interpretation is in all probability to be applied to the "nucleolo-centrosome" (Keuten) of *Euglena*, especially as Hartmann and Chagas (loc. cit.) have shown that promitotic division of the karyosome, by means of a centrodesmose, occurs in another Euglenoid, *Peranema trichophorum*.

behaviour of the karyosome at different periods of the life-cycle. For instance, as regards the Coccidia, speaking generally it may be said that during the schizogonic, vegetative phases, the karyosomatic chromatin becomes subdivided up, in a promitotic manner, amongst the daughter-individuals; on the other hand, as a rule, on the approach of the sporogonic part of the cycle—frequently during gametogony or else early in the history of the zygote—the karyosome is mostly eliminated, a “nuclear purification” of the unrequired, trophic chromatic material taking place.<sup>1</sup> Moreover, in connection with this view, a very simple explanation can be offered of the presence of an intra-karyosomatic centrosome, one which appears to me to render quite unnecessary the involved conception of the karyosome discussed above. It must be remembered that the promitotic type of division, which is the type found where the centrosome is contained within the karyosome, is of a primitive character, as its name implies. It is most likely that the reason why the centrosome, i.e. the intra-nuclear division-centre, is inside the karyosome in such cases is simply because the latter body does contain, for the time being, the larger proportion, or it may be nearly all of the chromatin of the nucleus, the division of which it is the function of the centrosome to bring about and regulate; in other words, because, having regard to the primitive character of the mechanism, the function of the division-centre is the better performed the more intimately it is associated with the chief chromatin-containing constituent of the nucleus.

Further, on this view a separation of centrosome and karyosome, as the nuclear development reaches a slightly more advanced stage, would be readily intelligible. Such an occurrence of the division-centre distinct from, or independent of, the karyosome (but at first, of course, remaining intra-nuclear) may have been brought about in more than one way. Thus it may be the result of a more elaborate develop-

<sup>1</sup> It may be noted that Léger and Duboscq (17), in their admirable account of the sexual processes among Gregarines, also adopt this interpretation of the elimination of karyosomatic material.

ment of the mechanism of division; an example of this is seen in the case of *Spongomonas*, to which reference has previously been made, where the centrosome passes out of the karyosome at the period of division and a definite mitotic figure is formed. Or, on the other hand, it may be due to the development of another type of nuclear structure, where, either during certain phases in the life-history or throughout the whole cycle, there is no longer a karyosome present in the nucleus as such, but the chromatin is more or less uniformly distributed on a reticulum throughout the general nuclear substance, in the middle of which the centrosome may persist.<sup>1</sup> And it is in this direction that the nuclear constitution has apparently developed in the *Hæmogregarines*. Lastly, a further stage in nuclear evolution would be reached by a combination of the two lines of development indicated, i. e. by the elaboration of the nuclear structure itself, associated with a more perfect development of the division-mechanism; and thus a condition might be arrived at such as is seen in the daughter-nuclei formed during the period of nuclear multiplication, which precedes gamete-formation, in many *Gregarines* (cf. the figures of Brasil [4], Léger and Duboscq [loc. cit.], Woodcock [39]), where we find perhaps the highest grade of nuclear constitution and mode of division attained among the Sporozoa.

<sup>1</sup> It is important to note that even where a division-centre is certainly present during particular phases of a life-cycle, this may nevertheless be wanting, or at any rate not recognisable, during other periods of the same life-cycle. Thus, in many *Coccidia* (e.g. *Coccidium schubergi*, *Cyclospora karyolytica*, according to Schaudinn), the division of the definitive nucleus of the zygote to form the sporoblast-nuclei is direct; but, on the other hand, in *Adelea* (cf. *A. ovata*, *mesnili* [Perez, 25] and *hartmanni*) the sporogonic divisions appear to be promitotic, i. e. more or less comparable to the schizogonic ones, allowance being made for the absence of a karyosome). Again, in the nuclear divisions of the sporont of the *Gregarine*, *Diplodina irregularis*, I have shown (39) that the first ones are direct (amitotic), the later ones mitotic.



We are now in a position to summarise, comparatively, the various types of nuclear condition which have been described in different *Hæmogregarines*. In *Karyolysus lacertæ* a definite karyosome is present in the youngest schizonts. This undergoes promitotic division which is usually unequal. The smaller half divides again, and the resulting portions ultimately become incorporated with the general nuclear material; the larger half of the karyosome, on the other hand, is eliminated from the nucleus and passes to the surface of the body-protoplasm, becoming altered and probably partially used up by the cytoplasm in its passage.<sup>1</sup> As already mentioned, I am of the opinion that the division-centre persists in the modified nucleus and can be seen at times as a definite central granule. I am unable to say whether a karyosome is developed again in a later phase of the life-cycle. In *Hepatozoon* (*Hæmogregarina*) *canis*, according to Wenyon (37), the karyosome persists throughout the schizogony, its division occurring in the usual promitotic manner; in this case, the body regarded by Wenyon as a karyosome is very small comparatively, and, I should say, represents little more than the intra-nuclear division-centre itself. Wenyon does not mention whether he observed any elimination of chromatic material before or during schizogony. On the other hand, in *Hæmogregarina nicoriæ* a karyosome cannot be distinguished at all, the nucleus appearing in all phases to have its chromatin more or less regularly distributed upon a reticular framework; a definite intra-nuclear centrosome is regarded, however, as being present. *H. stepanovi* shows, as I consider, a very interesting stage in the disappearance of the karyosome as a distinct organella. In certain phases a nucleolus is present,

<sup>1</sup> It is instructive to note that a similar elimination of karyosomatic material before the young schizont proceeds to nuclear multiplication is described by Averintzeff (1) in the case of *Barrouxia* sp., parasitic in *Cerebratulus*. The process may apparently take place according to one of two slightly different modes, the second of which furnishes a close parallel to the nuclear behaviour of the corresponding phase in *Karyolysus*.

occupying the same excentric or peripheral situation in the nucleus which is occupied in other forms (e. g. *Karyolysus*, "*Adelea*") by the karyosome. I suggest that this element represents the plastin basis of an ancestral karyosome, the chromatin which it originally stored having become now (permanently) distributed through the general nuclear material in the form of numerous large conspicuous grains.<sup>1</sup> In this connection an observation made by Reichenow is significant. He found that in the young growing schizont, chromatic substance is regularly eliminated from the nucleus and cast out of the cell-body of the parasite, i. e. a precisely similar occurrence to that seen in *Karyolysus* and *Barrouxia* sp. Reichenow is uncertain whether it is the nucleolus ("*Binnenkörper*") which is thus got rid of; but, as he himself points out, the fact that the nucleolus is always very faintly stained, while the expelled element stains on the contrary deeply and is manifestly chromatic in origin, is against this view. Moreover, I may point out that in slightly older schizonts again, the nucleolus is still present in the nucleus (c.f. Reichenow's figs. 73-75). Hence it is more probable that this eliminated chromatic substance is derived from the general nuclear chromatin. As this process here doubtless has the same object as the corresponding one in other parasites, the inference is that the chromatin which in other cases is stored up in the karyosome is in *H. stepanovi* incorporated with the rest of the chromatic material of the nucleus, the plastin basis of the karyosome alone remaining. On this explanation, and having regard to the views I have expressed above, it is readily understandable why the nucleolus does not divide, with the formation of a "*Hantel-Figur*," a fact which appears to have puzzled Reichenow. There is no need for a division-centre to be present in the nucleolus because it no longer possesses the chromatin of a

<sup>1</sup> So prominent are these grains and apparently in certain phases usually of a fairly constant number (i. e. within limits) that Reichenow is inclined to regard them as definite units comparable to chromosomes.

karyosome (chromatin-nucleolus). If, as seems to me quite possible, a centrosome does occur in *H. stepanovi*, this is most likely to be in the centre of the chromatic network of the nucleus.

Before concluding this section, I should like to add a few remarks about the nuclear condition seen in the piscine *Hæmogregarine*, *H. triglæ*, to make a comparison with which was my original intention in commencing to study the nucleus of *K. lacertæ*. Minchin and Woodcock (*loc. cit.*) found that in both the small forms and the two large types of the parasite one or two large grains are frequently, though not invariably present, situated either close to the nucleus, or some varying distance from it; these bodies are very deeply stained and prominent in films stained by iron-hæmatoxylin. The nucleus itself appears comparatively pale and consists of small grains of chromatin, often somewhat faintly stained, on an irregular network. In Giemsa-stained smears it is difficult to distinguish this grain (or grains) when close to the nucleus. In our paper describing *H. triglæ* we regarded these elements as not chromatic, but rather of the nature of centrosomes. The extra-nuclear position of the body, together with the fact of its being often paired, seemed to us very much against its representing a karyosomatic element. Moreover, the appearance of these grains after being stained with Twort's stain did not, in our opinion, furnish sufficient evidence in favour of their being chromatic. It is true that in freshly made preparations they were often stained red, i.e. with the neutral red, the chromatin staining constituent of Twort; but they had no strong affinity for the red, because in preparations which had been made some time the red tint had quite vanished from them, although the nucleus itself retained the red colour. I think we were misled by this behaviour after Twort. While it may be said that only chromatic elements are stained red by this stain, I think now that it is nevertheless quite likely that chromatin in some states or conditions may possess only very slight affinity for the neutral red.

Discussing at the time the question of the *Hæmogregarine* nucleus, we considered this to be of a distinct type, entirely lacking a karyosome. Börner (3), in his account of Reptilian *Hæmogregarines* (the best, as regards cytological details, which had been published up till then), had expressly stated that he never in any case found a karyosome present. Moreover, mentioning the matter in conversation with Miss Robertson, she also agreed that the *Hæmogregarine* upon which she was at the time working (*H. nicoriæ*) also had no karyosome associated with its nucleus. The only mention in the literature up to then of the occurrence of a karyosome in the nucleus of a *Hæmogregarine* was by Wenyon (36), in the case of certain phases of *H. gracilis*, from the liver of *Mabnia*. It appeared to us at that time highly probable that Wenyon had mistaken phases of some Coccidian parasite of the liver for phases of the *Hæmogregarine*, particularly as other, rather similar stages figured by Wenyon, which were undoubtedly referable to the life-cycle of *H. gracilis*, showed no karyosome in the nucleus. In the light of the observations discussed in the present paper, I willingly admit that our opinion was very probably mistaken, and that Wenyon may have been quite right in attributing all the phases he figured to the life-cycle of *H. gracilis*.

In short, it is now perfectly clear that the *Hæmogregarine* nucleus cannot be considered as being of a distinct type, but that, on the contrary, it shows close agreement with, or is easily derivable from, the Coccidian nucleus. Either a definite karyosome is present, at all events during some part of the earlier (schizogonic) phase of the life-history, when it behaves in a manner quite parallel to what is found in certain Coccidia, or else its complete absence is readily accounted for by a consideration of its behaviour as the development proceeds in those parasites in which it does occur.

Therefore, in the case of *H. triglæ*, it is most probable that the conspicuous grains also represent karyosomatic elements, and that they do contain chromatin in some form or other. In our preparations we did not observe any division-

figures, but it is not unlikely that where two grains are present, they have originated by the division of one, if comparison is made with the somewhat similar condition seen in *Kalyolysus* and "*Adelea*." Whether, again, a portion of the chromatic material is used to replenish the chromatin of the reticulum, or whether it is all unrequired and eliminated, I am unable to say. No definite centrosomic granule was noticed within the nucleus itself.

#### NOTE TO PART III.

Since this part was written my attention has been called to an important paper by Debaisieux (6A), on the *Coccidia* of *Lithobius*. I am only able here to indicate briefly the conclusions arrived at by this author, in so far as they bear upon the chief points which have been considered in the above section. Debaisieux also finds, as do Schellack and Reichenow, that phases of more than one parasite have been confused in previous descriptions of *Adelea ovata*. No reference whatever is made, however, to Schellack and Reichenow's note—an omission which is to be regretted. Debaisieux agrees that there is no double (or sexual) schizogony in the true *Adelea ovata*; but whereas Schellack and Reichenow refer those phases which do not belong to *Adelea* to *Barrouxia alpina*, Debaisieux refers them (at any rate, those observed by Jollos) to *Coccidium lacazei*. I am very pleased to find that Debaisieux also entirely upholds the occurrence of a true division-centre (centrosome) and of promitotic division of the karyosome, as described by Jollos (*loc. cit.*); though it may be mentioned that, as regards the precise modes of nuclear behaviour and division in the later stages of schizogony, he differs in certain points from that author. Further, Debaisieux takes a view upon the nature and significance of the karyosome quite similar to that which I have mentioned above; and this author also dissents from the ideas about the karyosome propounded by Hartmann and his school.

IV. THE NUCLEAR STRUCTURE OF *LEUCOCYTOZOON* AND *HALTERIDIUM*; THE SIGNIFICANCE OF THE SO-CALLED BINUCLEATE CONDITION IN THESE FORMS, AND ITS BEARING UPON THE AFFINITIES OF THE *HÆMOSPORIDIA*.

THE observation of the occurrence of a distinct karyosome in certain *Hæmogregarines* led me to study again, from this point of view, the much-discussed nuclear condition found in the gametocytes of *Leucocytozoon* and *Halteridium*. As is now well known, female individuals of both these parasites, when stained by some modification of the Romanowsky method, show besides the ordinary nucleus, which is stained red, another very definite nuclear body, which stains much more deeply than the other, and at times appears almost black; this additional chromatic element may be either close to (in contact with) or quite separate from the nucleus. In the case of *Halteridium* this body has, in female individuals, the form of a conspicuous grain, but in the distinctive individuals which have been regarded as neutral or "indifferent" (which, it may be incidentally remarked, seems to occur only rarely), it is even more prominent and may be almost as large as the nucleus. In the case of male individuals, however, I have not succeeded in making out anything comparable to this structure. As I have previously described and figured the appearance shown by *Halteridium fringillæ*, when stained by Giemsa, I need not refer further to it; I have found exactly the same appearance in *Halteridium noctuæ* of the little owl.

In the case of *Leucocytozoon ziemanni*, the celebrated *Leucocytozoon* of the little owl, the additional chromatic body is very large and prominent in the female gametocytes (Pl. 10, figs. 4-6), and by no stretch of imagination can it be regarded merely as a grain! Anything more like the trophonucleus and the kintonucleus of one of the large "blue" Trypanosomes present in the same bird might be expected to appear, in a resting, intra-cellular condition, it is impossible

to suggest; and I well remember that when I first saw such individuals in preparations, really well-fixed and stained,<sup>1</sup> I felt no more doubt that Schaudinn's view would prove to be correct than I felt about being at Rovigno. In my opinion, this remarkable resemblance was the foundation upon which Schaudinn built up his whole theory of the ontogenetic connection between the Trypanosomes and the intra-cellular parasites (*Leucocytozoon* and *Halteridium*) of the little owl. To return to *L. ziemanni*, in the male gametocytes the great majority here also show no chromatin body in Giemsa-stained smears besides the large, oval, diffuse nucleus, the scattered granules of which stain faintly a pale red (fig. 7). Occasionally, however, two or three small bodies or grains, which may differ slightly in size and which stain red somewhat more deeply than the nucleus, can be made out situated close together near the margin of the nucleus, forming as it were a clump almost in contact with it (fig. 9). These small structures are really only conspicuous in individuals which are if anything over-stained. Nevertheless the elements thus occasionally indicated in the nucleus of the male forms, stained by Giemsa, are found to be practically as constant in occurrence, in films stained by iron-haematoxylin, as is the single large body present in the female forms.

<sup>1</sup> This remark is not made with any idea of self-praise; it is by no means an easy matter to obtain *Leucocytozoon* well fixed and stained, even according to the Romanowsky method, so as to show the nuclear structure properly, and also the different parts of the host-cell, in their true form and relation to the parasite. It is only necessary to glance at many of the figures of different species of *Leucocytozoon* hitherto published to realise this. Either the parasites are hopelessly distorted and flattened out (cf. Dutton, Todd and Tobey's figs. [2]), or the only sign of a nucleus is a space-like area in the middle of the cytoplasm (as in some of Mathis and Léger's recent figures [3]); some of Wenyon's figures, too, of *L. neavei* (8) are far from giving an accurate representation of the form and nuclear details. My figures in the present paper, as also those of *L. fringillinarum* in a previous memoir (9), show approximately the true nuclear appearance, as will be seen when the condition found in wet-fixed preparations stained by iron-haematoxylin is discussed.



The study of these gametocytes of *Leucocytozoon* in films stained with iron-haematoxylin is most instructive. Berliner, in his account of Flagellates (1), has also given figures of the intra-cellular parasites, *Leucocytozoon* and *Halteridium*, stained in this manner, with the idea of showing that they agree with the Binucleata in the possession of two nuclei (i.e. the occurrence of nuclear dimorphism); he does not, however, give any description of the details of nuclear structure. As regards the female forms, the figures given by Berliner show, on the whole, the same appearance as that which I have found.

Taking a general view, as it were, first, of the nuclear structure of the female gametocytes (figs. 11-17), this is seen to be, in many respects, of a similar type to that of the young schizonts of "*Adelea*." For the most part the nucleus consists of a fairly large, central mass, which appears finely granular and stains to a moderate degree; surrounding this the same clear, almost colourless zone can usually be made out, which is present in "*Adelea*" (cf. figs. 1-3). Berliner figures well-marked rays traversing this narrow zone; now and again I am inclined to think I have caught a hint of the presence of one or two of these rays, but in my preparations they are so faint and elusive that it is difficult to be certain. Standing out conspicuously by reason of the intensity with which it stains is the large chromatic body, which is so prominent in Giemsa-stained smears; this is always spherical and generally surrounded by a distinct halo, as is the karyosome in "*Adelea*." It is usually in close association with the nucleus proper, though it may be distinctly separate from the latter, as in fig. 11, but I have never seen it so far removed as I have found it in Giemsa-stained preparations (cf. figs. 6 and 8). In two or three cases I have observed two such bodies, of unequal size, and neither so large as when there is only one, lying at opposite sides of the central mass (figs. 12, 17); the resemblance of the nuclear condition in such cases to that seen in figs. 1 and 2 of "*Adelea*" and figs. 24, 26, Pl. 9, of

Karyolysus, in a preceding note, is striking. This occurrence is apparently infrequent,<sup>1</sup> but the observation of it has considerably helped to influence me in my decision to relinquish, as no longer tenable, the view I have formerly held respecting the origin and significance of this much-discussed element. Regarding this body (or bodies) in the light of the nuclear constitution existing in certain phases of "*Adelea*," and especially bearing in mind the fact that I have myself made known above a similar karyosomatic condition in a blood-parasite, Karyolysus, the conclusion seems to be forced upon one that here also we have to do with a true karyosomatic element, and not, after all, with a body comparable to the kinetonucleus of a binucleate Flagellate.

In regard to the finer cytological points, the nucleus of these female gametocytes differs slightly from that of the early schizonts of "*Adelea*" and Karyolysus, as might indeed be expected when the different nature and subsequent development of the two types of individual is borne in mind. In those cases where there are two unequal-sized karyosomatic bodies (as I intend to designate these intensely staining elements in future), I cannot say whether they arise by the division of a single original one, in a primitotic manner, though I think this quite likely. I have not observed a spindle connecting them, but that may be because I have only found very few individuals in which there are two of these bodies. On the other hand, there is certainly a division-centre in connection with the central part of the nucleus, for not infrequently a distinct spindle (centrodesmose) is seen stretching between two granules, one of which stands out particularly from the more faintly stained chromatic material (figs. 11, 14). One of Berliner's figures also show this centrodesmose. The two granules connected by this spindle appear to be situated at the periphery of the central mass, and one is usually larger and more prominent than the other. In one instance I have observed a spindle running from the larger

<sup>1</sup> It is somewhat remarkable that, in Giemsa-stained smears, I have never noticed two of these structures associated with the nucleus.

granule to the karyosome (fig. 16). The larger granule may be present without there being any spindle or smaller granule (fig. 13). Very frequently close to this large granule is another one of about the same size and appearance; but this latter appears to lie always outside the central mass of the nucleus, at the outer edge of the clear, surrounding zone (figs. 14-16).

Turning now to the male gametocytes, there is always a large, oval nuclear area. As in Giemsa-stained smears, this is more usually very faintly stained (figs. 18-22)—remarkably so for a nucleus after iron-hæmatoxylin. It consists apparently of a loose reticulum with fine granules scattered throughout it. Here, also, this oval area is surrounded by a more or less distinct clear zone, but I have never, in this case, been able to make out any traces of rays crossing it, though Berliner (*loc. cit.*) just indicates a few in one of his figures purporting to be of male gametocytes. Berliner's figures of male individuals, however, are much less satisfactory than those which he gives of female ones; and, in fact, I am very much inclined to doubt their representing male forms at all, for reasons which I will mention shortly. In the majority of cases the outer limit of the nucleus, external to the narrow, clear zone, is more or less strongly impregnated with chromatin, in the form of distinct granules, which stain deeply, and in optical section constitute a prominent chromatic ring, sharply delimiting the periphery of the nucleus (figs. 20-23). It is noteworthy that this well-marked peripheral zone of chromatic granules in the male nucleus is apparently never to be observed in Giemsa-stained smears; it is not obvious in any of my preparations (for instance the individual of fig. 7 is on a smear made at the same time as the cover-slip preparation on which is the parasite of fig. 21), nor is it shown in any figures hitherto published. However, this zone is not always apparent, even in iron-hæmatoxylin preparations; thus the individuals of figs. 18, 19 do not show it. Although, of course, the intensity of staining and the degree of extraction

have a marked effect upon the prominence of this chromatic zone and the apparent size of the granules composing it, as equally upon the appearance of the host-cell nucleus (cf. figs. 22, 23), nevertheless I do not think the seeming absence of the zone in the instances mentioned is due, to any great extent, to the technique, i. e. to a less intense staining or to an excessive amount of extraction; for one thing, both the host-cell nucleus may be more intensely stained, and the host-cell itself, i. e. its spindle-like prolongations, more readily discernible, in cases where the nucleus shows no chromatic zone than in cases where it does (cf. figs. 18, 19, and 20, 22). Again, while all the preparations made from one infected owl may show the chromatic ring prominently, in those made from another bird this feature will be either not nearly so strongly marked, or else not discernible at all; this fact also points to a difference in this condition, in different cases or at different periods. I may emphasize the fact that I have never observed it in female gametocytes.

Almost constantly associated with the male nucleus is a group of small, spherical, deeply staining elements. Very generally these are three in number; a larger, more external one and two smaller ones, of approximately equal size. The larger body is situated at the edge of the nucleus, or just outside the border or periphery (figs. 18, 19), and is often surrounded by a distinct halo. Both in position and appearance this element agrees closely with the large, conspicuous body associated with the nucleus in the female gametocytes, the only apparent difference being that it is never so large; and I do not hesitate to suggest that it represents the same organelle in the male forms, namely a karyosome. Why this chromatic element should stain so much more easily and intensely with Giemsa in the case of the female individuals than it does in the male forms is another instance of the peculiar and misleading vagaries of this stain. The two smaller elements I have mentioned, which apparently represent a pair, are situated at about the limit of the central

diffuse area of the nucleus, i. e. just internal to the narrow, clear zone (figs. 18, 19); (of course the disposition of these various organellæ can only be correctly ascertained when they happen to lie in the plane of optical section). The two granules are sometimes connected by a short but distinct spindle (fig. 22); and in one case (fig. 21) I have observed a spindle joining one of these granules to the larger body (karyosome).

It remains now to compare these granules occurring in the male nucleus with those described above in the female nucleus. It is highly probable that the pair of granules in the male form corresponds to the two approximately equal-sized granules seen in the female gametocytes of figs. 14-16, near the periphery of the nucleus. There is a marked agreement, moreover, between the nuclear condition shown in figs. 21 and 16, of male and female individuals respectively, where the large karyosome is still connected by a fibril with one of the two granules. A distinguishing feature in all the cases I have observed is that in the female nucleus the paired granules are radially arranged, while in the male they are tangentially arranged. The condition seen in the female individual of fig. 11, where the inner of the two granules has undergone a further unequal division, a still smaller granule remaining connected with it by a distinct centrodesmose, apparently represents a later phase which I have not seen in a male gametocyte. An important question is: Are these paired granules to be regarded as constituting kinetic elements (centrosomes) solely, or as representing small karyosomatic elements (i. e. containing also chromatin)? That they contain a division-centre does not require to be emphasised, as this fact is clear from the various centrodesmoses I have described and figured in connection with them, in both male and female nuclei. In my opinion it may be regarded as certain that the very small peripheral granule seen, for instance, in fig. 11 is a centrosome (or centriole), still in connection with its fellow one; as, however, the body at the other end of the fibril is slightly larger, it may be, perhaps, that this latter

element is really a very small daughter-karyosome, possessing a certain amount of chromatin which encloses the true centriole (cf. the very small karyosome and centriole in *Hepatozoon canis*, see p. 203). If this be so, the other granule of the pair must also be interpreted as a small karyosomatic element, and, of course, also the corresponding pair of granules in the male nucleus.

To understand the exact significance of the somewhat complex system of divisions and resulting elements which I have described, a study of their behaviour during the further development, i.e. gamete-formation and fertilisation, would be necessary. From a consideration of figs. 14-16 it may perhaps be suggested that the more external of the paired granules, situated usually just outside the clear nuclear zone, represents a further elimination of unrequired nuclear material, possibly a kind of maturation-process; but I have no indication whether the same explanation holds good in the case of the male forms. Lastly, with regard to the large karyosome itself. Does this body contribute any of its store of chromatin to the general chromatic material during the growth of the gametocyte, or is it entirely eliminated as unnecessary? In this connection one point which I have noticed may be mentioned. The karyosome is slightly but distinctly larger in a male nucleus which does not show the chromatic zone than in one which possesses this feature (cf. figs. 18, 19, and 20, 22). This may possibly indicate, in the latter case, some augmentation or replenishment of the chromatin of the general nuclear substance and a corresponding diminution of the amount held by the karyosome.

It will be clear, I think, that in regard to the essential features the nuclear constitution of both male and female gametocytes of *Lencocytozoon ziemanni* shows a close agreement, and this notwithstanding the apparently pronounced differences shown when they are respectively stained by Giemsa. It is remarkable how constant in appearance, on the whole, the nuclear condition is found to be; and this fact adds, of course, to the difficulty of interpreting the elements



observed. While, however, the male and female nuclei of *Leucocytozoon* are fundamentally similar in type, there is no possibility of mistaking the one for the other, even in films stained by iron-hæmatoxylin, on account of the constant differences in detail. As I have already mentioned, Berliner's figs. 50 and 53, Pl. 29, which he regards as representing male gametocytes, do not agree at all with the characteristic appearance I have found and above described. The nucleus itself is figured as round, instead of being, as it almost invariably is, a pronounced oval in shape; and although it is somewhat larger than that of the female individuals which Berliner figures, it is nothing like the size which the male nucleus usually is. Moreover, the central area is stained more deeply, like that of the female forms, instead of being pale, even paler than the surrounding cytoplasm, as in the male forms; and lastly, there is no sign of the peripheral chromatic zone. The associated, intensely staining body is also very large, like the karyosome of the female gametocytes, and there is no indication of the small paired elements close to it. In short, I feel almost certain that the individuals figured by Berliner as of male sex are really also female forms (cf. his fig. 50 and my fig. 13, for instance).

I have dealt first with the nuclear structure of *Leucocytozoon* for two reasons: firstly, because in spite of its somewhat complex character it is not nearly so difficult to make out satisfactorily, on account of the large size of the parasites and the absence of pigment-grains, as is that of *Halteridium*, when fixed by a wet method and stained with iron-hæmatoxylin; and secondly, because it is more readily comparable with the nuclear condition found in the young forms of "*Adelea*" and *Karyolysus*. I have now to consider the nucleus of *Halteridium*, and will again begin with the female gametocytes. Berliner (loc. cit.) in the explanation of his figures of this parasite says nothing at all about the sex; so far as his figs. 58-60, of fairly large or adult individuals, are concerned, these certainly represent female forms. No male forms are figured, just as I maintain is the case with his figures of *Leucocytozoon*. The appearance



of the female gametocytes, according to Berliner, also agrees on the whole with the condition I have found. In most individuals the nucleus has a close resemblance to the characteristic flagellate type of nucleus. It appears as a very clear, round area, of relatively small size, which is sharply marked off from the surrounding cytoplasm and is most probably limited by a definite membrane; in the centre is a prominent, intensely staining karyosome (figs. 24-27). Berliner figures distinct rays passing from this central karyosome to the periphery of the nucleus. I certainly believe in the presence of these rays, serving, as it were, to sling the karyosome in position, but I cannot figure them for the simple reason that, even under the best optical conditions at my disposal, I am unable to actually see them myself; and I may say that others, who have kindly scrutinised several individuals on my preparations with this object, have also failed to discern them. Nevertheless I remember perfectly well once showing one of these preparations to my colleague Miss Robertson, then working in this laboratory, and she distinctly saw some rays in two or three cases, and sketched them for me. Hence, in the determination of these extremely delicate and difficult points one's own powers of vision are an important factor. Very frequently, at one side of the nucleus and usually close to, almost in contact with the membrane is a distinct granule, which is small and does not stain black so intensely (figs. 24-27). Now and again an obvious fibril or spindle connects this granule to the karyosome in the nucleus (cf. also Berliner's figures).

This was the nuclear constitution of *Halteridium* as I knew it when I wrote the postscript (à propos of Berliner's figures) to the paper by Minchin and myself (5) on the comparison of the nuclear structure of *Hæmogregarina triglæ* and *Trypanosoma raia*, and when I wrote the note on *Halteridium fringillæ* in my first study on Avian *Hæmoprotozoa* (9). It will be generally admitted, I think, that in view of the pronounced difference shown between this type of nucleus and that of *Hæmogregarines* (as the

latter was then known), when both were stained by a reliable cytological method, I was at the time quite justified in regarding the nuclear condition in *Halteridium* as corresponding closely to the karyosomatic type of nucleus seen, for instance, in a Trypanosome; and, further, in considering the definite, small associated element to represent a kinetonucleus in a "rückgebildet" condition as Berliner suggested. As a matter of fact, even until quite recently, and since I have realised the essential Coccidian nature of the nucleus of *Leucocytozoon*, I have been at a loss to explain this apparent resemblance of the *Halteridium*-nucleus to the binucleate condition and its difference from that of *Leucocytozoon*.

It is only within the last few weeks that I have learnt the true explanation of the matter and at last definitely settled, as I consider, the meaning of the nuclear appearance seen in *Halteridium*. The mistake has really been, I believe, in comparing the small associated granule, seen in films stained by iron-haematoxylin, with the conspicuous, deeply staining organella seen in Giemsa-stained smears, at any rate so far as regards the adult parasites. It so happens that some of my best iron-haematoxylin preparations of *H. noctuæ* are from an owl which had a heavy infection, and in which the great majority of the parasites were young, or intermediate-sized forms, relatively few being full-grown individuals. Looking over these at the time they were made, and again before writing the postscript above alluded to, I remember noting the general uniformity which was apparently presented by the nuclear structure. The small forms, the intermediate-sized ones and the few large parasites I came across all showed the karyosomatic type of nucleus, with or without the small accessory granule (and this is to be regarded, of course, as the regular condition, cf. figs. 24-27). As I then remarked, what I observed corresponded closely with what Berliner had figured. This being so, I did not undertake any systematic searching of these preparations at that time, as I wanted to continue first my study of the Avian Trypano-

somes. I naturally concluded that both Berliner and I myself had seen the same nuclear condition as that which I had considered to represent nuclear dimorphism when found on Giemsa-stained smears. I remember putting aside these wet preparations of *Halteridium* until a convenient opportunity for their detailed study should come along, with the thought that there was at least one point which was extremely difficult to determine from an iron-hæmatoxylin preparation, namely whether a particular individual was of male or female sex; it appeared to me as if, notwithstanding the well-marked distinction between the male and female nucleus after staining with Giemsa, the nucleus of both kinds of gametocyte was really of essentially the same form and structure, and the same view seemed to have been taken by Berliner, since he did not distinguish the sex.

Having found, however, since I began to study the cytology of *Leucocytozoon ziemanni*, that there is a constant difference between the nucleus of the male and female gametocytes respectively when stained by iron-hæmatoxylin just as in the case when stained with Giemsa, it was necessary to return to the *Halteridium* and try and settle the question as regards that form. Fortunately, I have recently obtained another chaffinch with a fairly good infection of *H. fringillæ*, in which most of the parasites are approximating to the adult condition and whose sex can therefore be readily distinguished. This time I at once made some iron-hæmatoxylin preparations, the examination of which happily enlightened me upon the whole question, in quite as great a measure as the study of Giemsa-stained ones helped to lead me astray in the first place. With the knowledge thus gained, I turned once more to my preparations of *H. noctuæ*, and have now been able to ascertain that the nuclear structure here also shows the same constant differences in the male and female forms.

In figs. 28, 29 are seen male gametocytes of *H. fringillæ*, and in figs. 30, 31 the corresponding forms of *H. noctuæ*. Both the red blood-corpuscles and the adult individuals of

the species of *Halteridium* infecting them are distinctly larger, it will be noticed, in the case of the little owl than in the case of the chaffinch. Hence the cytological details can be made out with somewhat less difficulty in the gametocytes of *H. noctuæ*, though of course not nearly so readily as in *Leucocytozoon*. It happened very fortunately that in one of my infected owls, the Halteridial parasites possessed, for some reason or other, very little pigment; many of my figures are drawn from this series of preparations, because in such a case there is no possibility of confusing the nuclear elements with pigment grains.<sup>1</sup> As is apparent from the figures, the nuclear structure agrees closely with that of *L. ziemanni*, and therefore a detailed description is unnecessary. As regards the large, oval, pale nuclear area in the male forms, I have never observed any indication of the peripheral zone of deeply staining chromatic grains, which are often so prominent in *L. ziemanni*; whether this is because they are not developed in the male nucleus of *Halteridium*, or merely because I have not succeeded in getting them to stain, I cannot say. There is, however, the same small, spherical, peripherally situated karyosomatic body, which now and again can be distinctly seen to be surrounded by a clear halo (fig. 30); and, close to it, the same dumb-bell shaped or else double centrosomic element.<sup>2</sup>

Turning now to the female gametocytes, it was the observation of the large, adult parasite (*H. fringillæ*) drawn in fig. 32 which suggested to me the explanation of the difference generally to be seen between the female nucleus of *Halteridium* and that of *Leucocytozoon*. In the

<sup>1</sup> It is perhaps scarcely necessary to say that this rather unusual feature does not imply that the nuclear details themselves differ at all from the condition found in other cases, where the parasites have the usual supply of pigment grains; the nuclear structure is obviously quite similar in my figures of *H. fringillæ*, which show numerous grains.

<sup>2</sup> In the case of *Halteridium*, these granules are so minute that it is difficult to believe they can be anything but the actual centrioles themselves.

individual figured, the conspicuous karyosome no longer occupies a more or less central position within the nucleus, but has passed distinctly to the outside, and bears apparently the same relation to the general nuclear substance as does the karyosome of the female individuals of *L. ziemanni* drawn in figs. 13-16. The chief points of difference to be noted are that the nuclear substance is here so faintly stained that it appears more like a spherical space than a nucleus; and secondly that I cannot (in this particular instance) make out any centrosomic granule. A similar condition is seen in figs. 33-35 of *H. noctuæ*, but two of these parasites show a distinct centrosome which is apparently intra-nuclear, though it may, of course, be lying near the upper or lower surface. The nuclear condition in this case agrees very closely with that of the female gametocyte of *L. ziemanni* drawn in fig. 13. I have not observed a single instance, however, where there are two granules in connection with the female nucleus of *Halteridium*, such as I have described as of frequent occurrence in *Leucocytozoon*. It is most probable, I think, that the centrosome<sup>1</sup> seen in figs. 33, 34 is the same element as that situated at the limit of the nucleus in figs. 24-26, but I have not found it connected by a fibril to the karyosome, where the latter has passed to the outside of the nucleus; the fibril perhaps disappears when the karyosome changes its position.

There can be no doubt, I think, that the smaller, intensely staining nuclear body in *H. fringillæ* (as seen when the parasites are stained by Giemsa), which I originally regarded as representing a kinetonuclear element, corresponds, not to the small peripheral centrosomic body seen in iron-hæmatoxylin preparations, when the nucleus has the condition shown in figs. 24-26, but to the karyosome, when this has passed to the limit of, or outside the nucleus (as

<sup>1</sup> In the case of the female nucleus also, I think it is preferable to regard this single granule as a centrosome only. I have not observed any secondary divisions or any further elimination (?) of small karyosomatic portions, as in the female nucleus of *Leucocytozoon*.

in figs. 33–35); at all events so far as large or adult individuals are concerned. That this is really the case is borne out by a fact which I noticed several times, namely, that only a certain proportion of the larger female forms of *Halteridium* (more, I should say, in *H. fringillæ*, fewer in *H. noctuæ*) show this characteristic additional element, in Giemsa-stained preparations; whereas practically all the female individuals of *Leucocytozoon ziemanni* exhibit it. We arrive, therefore, at the important result that when the female nucleus of *Leucocytozoon* is compared with that of *Halteridium* in the same phase, the two are found to be of essentially the same type of structure. Their apparent dissimilarity, as frequently observed, is due to the fact that in *Halteridium* the karyosome retains its central position within the nucleus throughout the period of growth of the gametocyte, and does not pass to the outside until the parasite is full-grown. On the other hand, in *Leucocytozoon* the karyosome appears to be always at the edge of, or else outside the nucleus, even in young or intermediate-sized individuals; I have never seen it within the central nuclear mass. This expulsion of the karyosome, which doubtless represents here, as in other cases, an elimination of unrequired chromatic material or “nuclear purification,” thus takes place very early in the development of the macrogametocyte of *Leucocytozoon*, but only at a comparatively late stage in that of *Halteridium*.

The facts I have observed and described above finally settle, in my opinion, the question of the connection of *Halteridium noctuæ* (and equally, of course, of *Leucocytozoon ziemanni*) with *Trypanosoma noctuæ*. It appears to me that these parasites have no direct connection whatever, either ontogenetic or phylogenetic. As readers of my first study on avian parasites (loc. cit.) will be aware, I felt then compelled to relinquish the view that *Halteridium* and *Trypanosoma* were phases of one life-cycle, though I still considered that *Halteridium* was to be derived from a *Trypanosome*-like parasite, which had become permanently

intra-cellular, in view of its possession (as was then thought) of the binucleate condition and of a typical Flagellate, karyosomatic type of nucleus. There may be some among those who uphold the locomotor or kinetic view of the karyosome who will even yet be inclined to say, Why should not the conspicuous, deeply staining body associated with the nucleus in *Leucocytozoon* and *Halteridium* still be regarded as representing a kinetonuclear element, perhaps in a "reduced" or non-functional condition?

The following are very strong reasons, I consider, against maintaining any longer the view that these parasites do exemplify the binucleate condition, as it is found, for example, in the case of a *Trypanosome*. In the first place, as I have shown in the preceding section (Note III of this series), the typical karyosome cannot be considered as a "locomotor component" at all; there is no evidence whatever that the karyosome itself stands in any special relation to the kinetic activities. Secondly, from the comparison of the true nuclear condition occurring in *Leucocytozoon* and *Halteridium* with that obtaining in the *Hæmegregarine*, *Karyolysus lacertæ*, and in certain phases of different *Coccidia*, it seems evident that the so-called kinetonuclear element in the first-named forms represents in reality the karyosome of these other parasites. Lastly, but by no means of least importance, when *Halteridium* and *Leucocytozoon* apparently show nuclear dimorphism, according to Giemsa-stained preparations, the nucleus itself is seen in films stained by iron-hæmatoxylin to be no longer of the well-known karyosomatic type, i.e. not comparable to the trophonucleus of a binucleate Flagellate; in short, as is clear from the study of my figures of *Halteridium*, the prominent extra-nuclear body is the karyosome of the nucleus.

The association of *Halteridium* and *Leucocytozoon* (and also, in all probability, of *Proteosoma* and the malarial parasites) along with the *Hæmoflagellates* in the group *Binucleata* has therefore to be given up. These *Hæmosporidia*, equally with the *Hæmogregarines*, must be regarded



as closely allied to the Coccidia; it seems to me now that there is no longer any reason for supposing that they are derived from a binucleate form, such as a Hæmoflagellate. It has been a great disappointment to me to find that the view so elaborately worked out by Schaudinn and apparently so firmly based on facts, which I in common with many other Protozoologists adopted enthusiastically, has had to be abandoned, step by step, until the entire edifice is seen to be without any true foundation whatever. From my own work I feel persuaded that the principal if not the only basis upon which Schaudinn built was that which I have above indicated, namely the remarkable resemblance between the nuclear condition of the female gametocytes of *Halteridium* and *Leucocytozoon*, when stained by the Romanowsky method, to that which a *Trypanosome* might be expected to show if in a resting phase. I greatly doubt, indeed, whether Schaudinn ever saw the nuclei of these gametocytes stained by iron-hæmatoxylin; certainly no figures of individuals so stained are given in the recent published collection of his works (7). From the study by Minchin and myself (4) of this question, more especially from the standpoint of the *Trypanosomes*, and also from the present study of the cytology of the intra-cellular parasites, it must be admitted that no real evidence of any kind can be found to support Schaudinn's view.

#### ADDENDUM.

In view of the publication quite recently of a paper by Prowazek (5A), on the "Geschlechtsdimorphismus der *Trypanosomen*," I feel obliged to add a few remarks to this note. Prowazek still maintains Schaudinn's view that *Leucocytozoon* and *Halteridium* represent, in each case respectively, merely the sexual phases of a *Trypanosome*. He thinks that Mayer ('Arch. Protistenk.,' xxi, 1911), has sufficiently proved this idea in the case of *Halteridium*, and he himself endeavours to show that an actual connection

exists in the case of the Leucocytozoan and Trypanosome parasites of fowls (Sumatra). So far as regards Mayer's account of the development of Trypanosomes from Halteridia, I shall have to criticise this in a later memoir; here I must confine myself to a brief consideration of the above-mentioned paper by Prowazek.

In the first place, it is impossible not to comment upon the appearance presented by the parasites in the figures on Prowazek's plates. I have pointed out above how frequently the figures hitherto given of Leucocytozoon have represented poorly fixed or stained specimens; but I do not recollect ever having seen any which are quite as bad as some of those on the plates in question. Speaking for myself, it is no exaggeration to say that, from many of the figures, taken by themselves, it is impossible to tell what they are meant to represent, so dreadfully are the parasites distorted and disorganised. It is obvious that no conclusion or interpretation can be accepted which is based upon preparations such as those from which these figures are taken.

Minchin and Woodcock (4), in their paper published only a month or two before Prowazek's appeared, and which presumably that author had not seen, have fully discussed the subject of the possible connection of Leucocytozoon ziemanni and the trypanosome of the little owl—the very parasites, i. e., on which Schandinn worked—but it is not out of place to repeat here the main conclusions at which we arrived. In spite of numerous and prolonged living examinations we never observed the least sign of the passage from one form into the other—in either direction; nor is there the slightest evidence to this effect in any of our permanent preparations. The better fixed and stained these are, the more closely the Leucocytozoon agrees in form with the appearance presented in the living condition; it is remarkably uniform, and scarcely varies at all.

I may mention here a point which I have not referred to in the preceding pages of this note with regard to the true

nature of the characteristic spindle-like prolongations invariably found in connection with the fully grown forms of *L. ziemanni* (and some other species). While I still held the view that this parasite (as also *Halteridium*) was a Binucleate and phylogenetically derivable from a Trypanosome-like form (9), I thought it most probable that, at all events in the proximal portion of these prolongations, there was some ectoplasmic layer belonging to the parasite which helped to produce the prolongations. In the case of species where the infected leucocytic host-cell remains rounded and does not develop any horn-like prolongations (as in *L. fringillinarum*), I regarded the ectoplasmic layer as having been completely lost. However, in my cytological study of *L. ziemanni*, the results of which have been given above, I have found nothing to support the presence of any ectoplasmic layer. In properly stained individuals (whether stained by Giemsa or by iron-haematoxylin) there is no real distinction or differentiation to be made out between the most proximal region of these prolongations, i. e. nearest to the more deeply staining cytoplasm of the parasite, and the distal portions towards the tip. In the great majority of cases the staining (which is always pale) is quite uniform in tint, only becoming gradually fainter as the prolongation narrows to its extremity (figs. 5-7, 10-12, 18, 22). Very occasionally, in Giemsa-stained smears, a space-like area can be seen, which is probably more or less artificial. In short, these prolongations undoubtedly represent solely the altered and extended cytoplasm of the infected leucocyte, this characteristic change being caused by the stimulus of the invading parasite as it grows. I gather that Wenyon (8) was inclined to this view in his account of *L. neavei*. An additional reason in favour of it is supplied by the facts in regard to the nuclear structure which I have made known, which indicate the essentially Coccidian nature of *Leucocytozoon*, since the *Coccidia* lack, of course, any differentiated ectoplasmic layer.

To refer now to certain other points raised by Prowazek,

this worker considers that he obtained evidence which pointed to a Trypanosome enveloping a red blood-cell and proceeding to "take up" its nucleus. As we emphasized in our paper, we searched in vain, time after time, for signs of such a metamorphosis; the utmost we found to occur was the attachment merely of a Trypanosome to an erythroblast or a uninuclear leucocyte by one extremity, which might be either the flagellar or the aflagellar one. Two features very difficult to explain on the assumption that a Trypanosome thus passes into or becomes a Leucocytozoon are (A) the fact that the latter parasite, in its well-known form, shows always male and female individuals, whereas a similar distinction cannot be made out in the case of the Trypanosomes; and (B) the fact that quite small intra-cellular Leucocytozoon parasites occur, which certainly grow up into the characteristic adults, since intermediate-sized forms can be found. With regard to the remarkable phenomenon described by Prowazek of a parasite (a so-called "agamont") becoming separated from its original host-cell, but taking a part of the nucleus and the cytoplasm of the latter with it and penetrating with these elements into a fresh host-cell, I can only say that in all my experience I have never seen anything which could in the remotest degree suggest such an occurrence. Prowazek's figs. 2-5, pl. i, are supposed to show different stages in this process; but I cannot gather anything of the sort from them. Lastly, Prowazek also considers that the gametocytes of Leucocytozoon undergo a division, usually into two, which is considered to be longitudinal and thus to indicate the Trypanosome-character of these forms. The author says that both male and female forms may so divide, but adds the very significant remark that in some cases the two resulting individuals are not of the same sex, but one is male and the other female. From Prowazek's figures 8-17, it is perfectly clear that we have really to do here with the same condition which I have described in *Halteridium* (10), and which had been previously found in *Hæmocystidium*. As I discussed in that note, there can be little or

no doubt that it is a question of a double infection of the host-cell, i.e. by two small individuals which may be of the same or of opposite sex, which grow up in contact with each other. And I am practically certain that the same explanation holds good also in the present case of *Leucocytozoon*.

In conclusion, I am sorry to say that, in my opinion, Prowazek does not bring forward a particle of reliable evidence which is of any use towards rehabilitating Schaudinn's unfortunate view.

THE LISTER INSTITUTE;  
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## EXPLANATION OF PLATES 9 AND 10.

Illustrating Dr. H. M. Woodcock's "Notes on Sporozoa. II-IV."

### PLATE 9.

[All the drawings are magnified 2000 times linear. All the figures, with the exception of figs. 1, 23, 41-43, relate to *Karyolysus lacertæ* (Danil.). Figs. 1-18 are from preparations stained with Giemsa].

Fig. 1.—Uninfected red blood-corpuscle.

Figs. 2-8.—Young parasites, with nucleus in the earlier phase, near the middle of the body.

Fig. 3.—A free individual, which has not yet penetrated a blood-corpuscle.

Figs. 9-18.—Older forms, with the nucleus in the later phase, situated (except in fig. 14) near one end of the body.

Figs. 14 and 15.—U-shaped forms.

Figs. 16-18.—Stout, bean-like individuals, resulting from the fusion of the two arms of the U. In figs. 17 and 18 the cytoplasm of the host-cell is not visible, and the host-cell nucleus forms a "cap" round one or both ends of the parasite.

[Figs. 19-43 are from wet films, stained with iron-hæmatoxylin.]

Fig. 23.—Uninfected red blood-corpuscle.

Figs. 19-22, 24-29.—Younger parasites, showing the karyosome (or else two karyosomes) closely associated with the nucleus.

Fig. 20.—Individual showing the promitotic division of the karyosome, the two halves being still connected by a spindle.

Fig. 21.—Small, free individual.

Figs. 30-40.—Older individuals, with the nucleus near one end of the body. In figs. 31, 37, a karyosome is still associated with the nucleus.

Figs. 32-36.—Parasites showing different stages in the alteration and gradual disappearance of the unused karyosomatic material.

Figs. 30, 39 and 40.—Individuals showing no sign of the karyosome or its remains.

Figs. 37 and 38.—Individuals surrounded by a distinct envelope, and in connection with which nothing whatever can be seen of the cytoplasm of the host-cell.

Figs. 41-43.—Merozoites or very young schizonts of "*Adelea ovata*" (*Barrouxia alpina*, according to Schellack and Reichenow).

Fig. 41.—A single large karyosome is present.

Fig. 42.—The karyosome has divided into two by a promitotic division, the connecting fibril being still present.

Fig. 43.—Four karyosomes of unequal size are present, resulting from further division.

#### PLATE 10.

[All the drawings are magnified 2000 times linear. I am indebted to Miss Rhodes for kindly drawing figs. 4 and 7. Figs. 4-10 are from Giemsa-stained smears; all the others from iron-hæmatoxylin stained films.]

Figs. 1-3.—Merozoites or very young schizonts of "*Adelea ovata*"

(*Barrouxia alpina*), showing two or more karyosomes in connection with the nucleus.

Figs. 4-6, 8.—Female gametocytes of *Leucocytozoon ziemanni*. (The parasite of fig. 4 is slightly flattened out.) *c.* General cytoplasm of parasite, containing its nucleus, and the associated karyosome. *h.c.* Cytoplasm of host-cell (leucocyte), prolonged into two tails or horns. *n.* Nucleus of host cell, elongated and dumb-bell shaped.

Figs. 7, 9 and 10.—Male gametocytes of *L. ziemanni*. Fig. 7 shows the general appearance of the nucleus, figs. 9 and 10 a much less common appearance. (The parasite of fig. 7 is slightly flattened out.) Lettering as in fig. 4.

Figs. 11-17.—Female gametocytes of *L. ziemanni*, showing details of nuclear structure. (To save space, in many cases only the middle portion of the parasite and of the elongated host-cell nucleus are shown.)

Figs. 18-23.—Male individuals of *L. ziemanni*, to show the details of nuclear structure. (In some of these figures also the spindle-like prolongations of the host-cell are omitted.)

Figs. 24-26.—Small, intermediate-sized and fairly large female individuals of *Halteridium noctuæ*, to show the nuclear condition as generally seen.

Fig. 27.—Large female form of *H. fringillæ*; similar nuclear condition. (Note the much smaller size of both blood-corpuscle and parasite in this case.)

Figs. 28 and 29.—Large and fairly large male gametocytes of *H. fringillæ*, to show the nuclear condition.

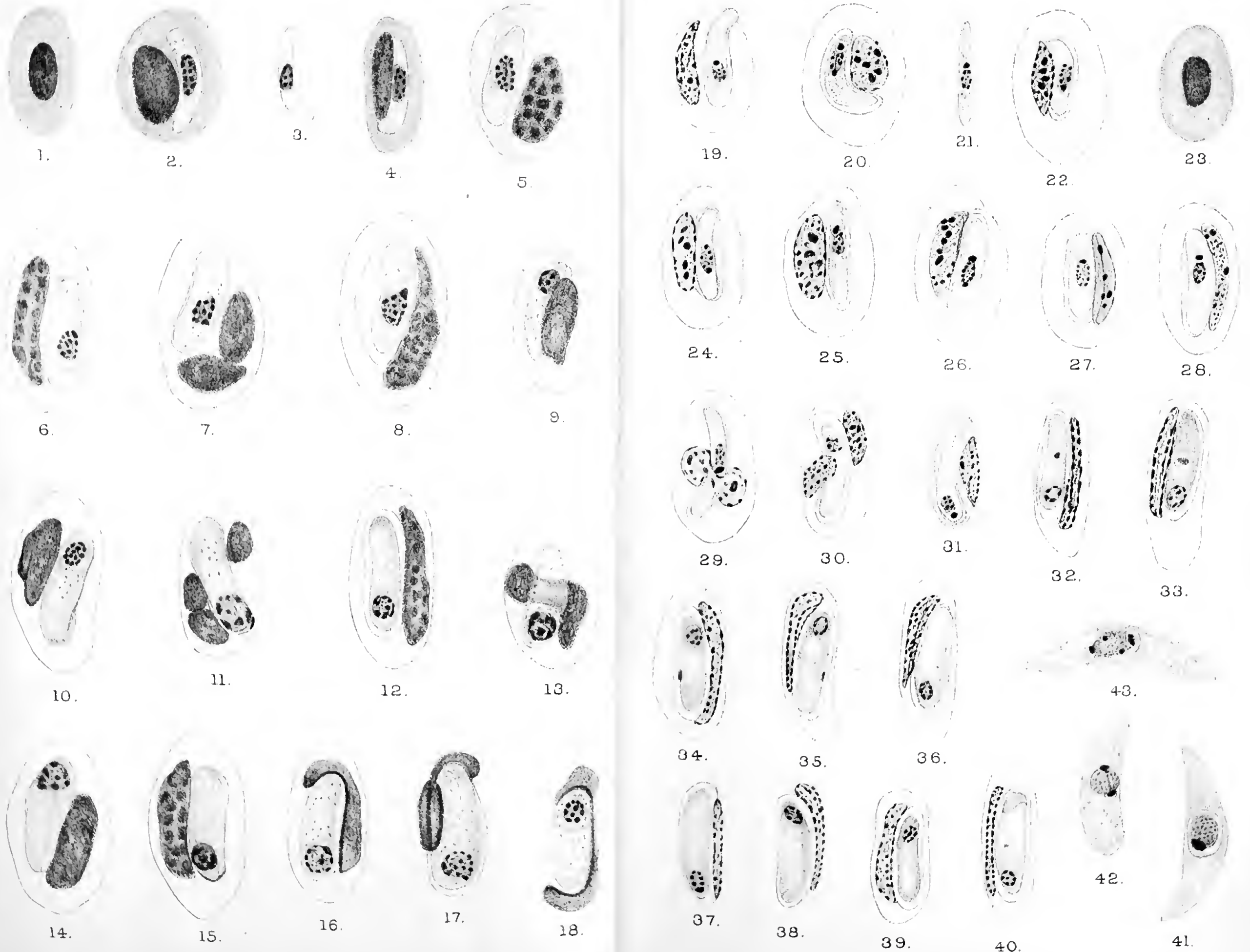
Figs. 30 and 31.—Ditto of *H. noctuæ*.

Fig. 32.—Large adult female individual of *H. fringillæ*, to show the extra-nuclear karyosome corresponding to the usual condition seen in *L. ziemanni*.

Figs. 33-35.—Large or fairly large female forms of *H. noctuæ* showing a similar or almost similar nuclear condition.





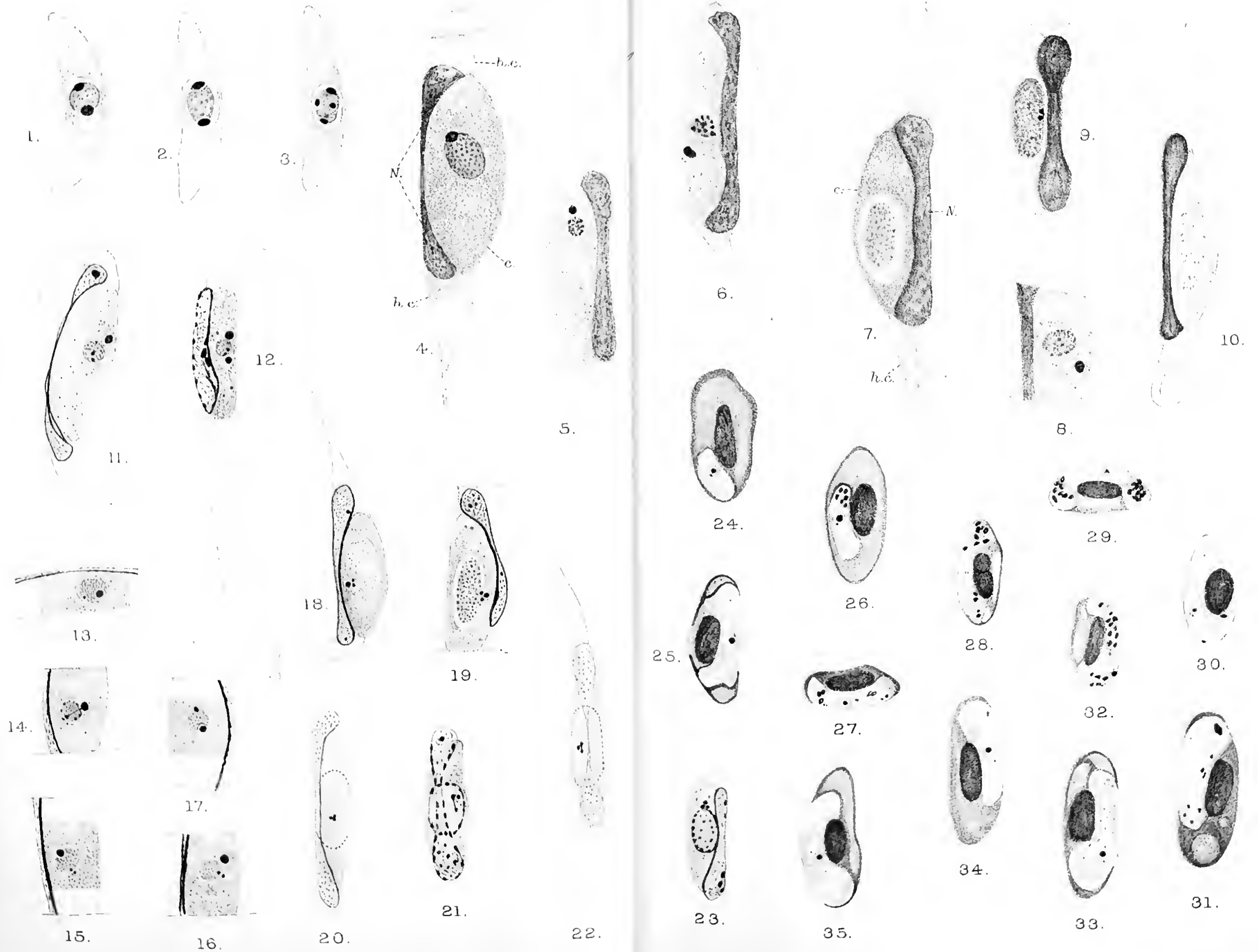














## The Dorsal Vibratile Fin of the Rockling (*Motella*).

By

**J. Stuart Thomson, Ph.D., F.L.S., F.R.S.E.,**  
Lecturer and Senior Demonstrator in Zoology, University of  
Manchester.

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With Plate 11.

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Most collectors on our shores are familiar with the rockling, and have observed the series of free, vibrating rays situated slightly anterior to the ordinary dorsal fin. It is the object of the present paper to give an account of this vibratile fin, together with co-related parts, and to indicate its function as a whole.

Bogoljubsky holds that this vibrating fin has not any physiological function other than that of a "lure," which is supposed to act in a somewhat similar manner to the anterior filament in the fishing frog or angler-fish, *Lophius piscatorius*. This explanation does not appear to me satisfactory, especially as no suggestion is made as to the precise method by which this supposed "lure," situated some distance posterior to the mouth, acts. From the standpoints of morphology and physiology I have come to the conclusion that the part has to be regarded as a highly efficient gustatory or food-detecting and food-locating organ.

The two species of rockling most commonly collected on our shores, and on the bottom in deeper water, are the three-bearded rockling, *Motella tricirrata*, and the five-bearded rockling, *Motella mustela*. As regards the habits of these fish, one may notice that they are shy, nocturnal, phlegmatic,

non-predaceous, and, as was pointed out by Bateson some years ago, do not, as a rule, seek or find their food by sight. On the sea-shore, the rocklings are mostly found lurking under stones between tide-marks, a large part of their skin being frequently coated with small grains of sand.

In connection with my contention that the vibrating fin is not purely and simply a lure, it is important to notice that the food of the rockling consists of crustaceans such as prawns and gammarids, annelids, star-fish, pycnogons, and even other fish.

The dorsal vibrating fin is very conspicuous, and in the still water of an aquarium its movement may be observed at a distance of three to six feet. The dimensions of the fin naturally vary considerably in specimens of different size. In a large specimen of *Motella tricirrata*, measuring 260 mm. in length and 95 mm. in girth, the groove in which the vibrating rays are situated was 32 mm. in length, and the large anterior ray was 7 mm. long. The fin is situated a slight distance (about  $\frac{1}{2}$  cm.) posterior to the head, and is separated from the ordinary dorsal fin by about an equal interval.

The vibratile fin consists of a series of comparatively small processes, which are almost continuously in rapid vibration, and anterior to these, a ray which is much longer and thicker than the others, and has much less power of movement. The individual rays are connected with one another near the base by a fold of skin, which passes off almost at right angles, and by this arrangement each ray has the power of independent movement. The rays arise medially from the base of a groove, the sides of which are chiefly formed by the lateral myomeres.

The vibration of the rays resembles the movement of cilia, and there is an independent motion of each, and a collective action of groups of rays. A sinuous wave-like vibration is thus produced, which drives currents of water in a latero-posterior direction over the sides of the groove. The movement of the rays or processes is almost constant, although it

may, at rare intervals, cease for several seconds, or even a few minutes. This interval of cessation is irregular and non-periodic. When the movement is about to be discontinued, the posterior rays cease first, and then later, those situated more anteriorly. In a similar manner, when the movement is being resumed, the anterior rays come into motion previous to the posterior processes.

In a good light the individual processes can be detected with the naked eye originating from a greyish ridge at the base of the groove, and with a reddish area on either side of the raised part. The anterior ray has only a very slight vibration in a lateral direction, which may be observed with a lens. The movement of the other processes is in a latero-posterior direction. On firmly pressing the surface of the anterior process with a sharp pencil or other small object it becomes depressed, and the movement of the remaining rays ceases, and conversely, on placing pressure on the small processes situated immediately posterior to the large ray, the latter is depressed. This depression of the anterior ray also occurs with fairly firm contact on the posterior processes, but not in such a marked degree. When a thin strip of paper is placed along the posterior surface of the large process, the small rays cease their movement; this means of communication seems localised, for when the paper is placed in contact with the anterior side, the cessation of vibration does not take place. There is thus apparently a certain degree of continuity between the large anterior process and the smaller rays. The small rays or groups of those have, however, the power of independent movement, for on touching certain of them their movement ceases, while those situated anteriorly or posteriorly still have the power of vibrating.

The result of coating the sides of the groove, in which the fin is situated, with black asphaltum, was a cessation of the movement of the vibrating rays for some time, although when the rockling was touched and consequently changed its position the rays recommenced to act; but otherwise the fish appeared curiously inert, almost as if asleep. In half an



hour's time a layer of mucus had become deposited on the sides of the groove.

On adding chloroform to the water in which the rockling is living, the movement of the vibratile fin ceases, although the animal freely moves its pectoral fin. Similarly, the addition of cocaine to the water containing a small rockling (2-3 in. in length) has the effect of stopping the movement of the rays for intervals varying from forty seconds to three minutes.

The action of the vibrating processes apparently maintains a clear area of skin on either side of the groove. My experiments, which consisted in letting down granules of carmine by means of a pipette, show that while these grains readily adhere to most parts (probably from the secretion of mucus), yet there remains an area on either side which keeps clear of carmine particles. The absence of carmine particles soon delineates very distinctly the area of vibration from the pigmented skin of the general surface of the body. A similar result evidently occurs when the fish is living in its natural habitat, as I have repeatedly noted that when rocklings are brought in from the shore they have a coating of sand on the general surface of the skin, with the exception of the area immediately surrounding the vibratile fin.

When the vibratile fin ceases movement, the large anterior process is folded backwards over the smaller processes, and these in their turn over those situated more posteriorly.

On lightly touching the sides of the groove, the processes fold themselves down on the opposite side of the groove to that which has been touched.

In the light of my subsequent remarks, it is interesting to notice that contact on the barbules and pelvic fins results in renewed movement of the vibrating rays when this has been stopped, but that the same result does not take place on contact with the pectoral fins. We will later notice that the barbules and pelvic fins have the same general mode of innervation as the area under discussion.

Bogoljubsky coated the rays from the dorsal surface with

gelatine and tannin, and even cut them away entirely, without apparently causing any physical discomfort, and without affecting the movements of the fish. He also states that cutting away the rays was subsequently followed by their regeneration. I obtained somewhat similar results so far as regards the fin itself, but I hold that the author has neglected the study of the skin in the immediate proximity of the vibrating rays.

In the course of my work I was early struck by the fact that the skin in the near neighbourhood of the vibrating processes is extremely sensitive; thus, for example, when a thin strip of paper is laid on the sides or apex of the groove bordering the processes, the movement of the latter is brought to a standstill. On the other hand, if the same contact is tried on the dorsal and pectoral fins, or on the surface of the head, the movement of the rays continues uninterruptedly. I will have occasion later on to refer to the occurrence of a number of tactile nerve-endings on the skin of the groove in which the rays are situated.

It is not easy to understand how Bogoljubsky arrived at his conclusion that neither from morphology nor from physiology can one ascribe any physiological rôle to this organ.

The vibratile fin of *Motella*, which may be termed the oral fin in contrast to the longer dorsal or aboral fin, is comparable in general structure to that of the unpaired dorsal fin of other *Gadidae*.

In the case of the smaller processes the structure is as follows:

From the spinous process of the vertebra a ray-carrier or radial passes dorsalwards, and at the apex of the latter a small spherical articulating process is situated to which the ray is attached. The radials or ray-carriers have a pillar-like form, differentiated into three parts, namely, a head, neck and base. The ray-carrier consists of hyalin cartilage which is richly impregnated with salts of lime. The individual ray-carriers are connected with one another by ligamentous tissue.

Each of the rays, with the exception of the first, contains two horny fibres which are separated from one another by connective tissue, and approach one another near the base. Each ray has an anterior and posterior basal enlargement. From the anterior of those a muscle passes almost vertically in a ventral direction and attaches itself to the lower part of the ray-carrier. This muscle acts as an erector elevating the ray. On the other hand, from the posterior process, a muscle passes obliquely downwards along the neck of the ray-carrier and fixes itself slightly dorsal to the attachment of the anterior muscle. This muscle acts as a depressor, lowering the processes into the groove.

The form of the first anterior process differs slightly from those just described. It contains only a single horny fibre, which divides at the base, and there are three small, basal processes to which the tendons of muscles are attached. It also differs from the smaller processes in that the ray is more directly connected with the ray-carrier by means of embryonal cartilage.

Transverse sections through the rays near their apices show an external, many-layered epithelium containing mucous glands; within this a deeply pigmented layer is situated, which surrounds a mass of centrally disposed connective tissue. The latter contains blood-vessels and two horny fibres.

The individual rays are, as previously stated, connected with one another by a fold of skin near the base.

The vibratile fin is supported in its position by means of a ligament, which surrounds and covers the spinous processes of the vertebræ, and dorsally to these also envelopes the centrally situated ray-carriers. This paired ligament takes its origin in the supra-occipital, and it proceeds in a posterior direction on either side of the ray-carriers, but on arriving at the aboral fin the two layers, right and left, unite into one.

The fin is, as previously stated, situated in a groove, whose internal boundaries are chiefly formed by the very prominent

lateral myomeres. The walls of this groove more or less protect the rays, especially when these are depressed. While I find myself in essential agreement with the description and figures of the structure of this fin as given by Bogoljubsky (pp. 329-332, figs. 3-7), I must now proceed to important points, more especially in regard to the structure of the surrounding skin, which are not dealt with in his investigations.

Fig. 2 illustrates the structure of the ventral part of the vibratile fin and adjoining parts, as seen in transverse sections. The skin covering the groove in which the fin is situated is scaleless, and its detailed structure is of the greatest importance for the understanding of this organ. The skin consists firstly of a many-layered epidermis, consisting of a series of squamous cells externally, which gradually pass over into more columnar cells internally. Within the latter a deeply pigmented layer is situated. The epidermis also contains numerous mucous glands, and, of more importance, a large number of tactile nerve-endings and terminal or taste-buds. Beneath the epidermis there is a slight space traversed by strands of connective tissue, through which nerve-fibres pass. These strands of connective tissue pass internally into a well-defined layer of compact, ligamentous tissue. Underneath the dermis the large lateral myomeres are situated. The medial and ventral part of the section also contains a mass of connective tissue with two nerves on either side, the larger one being the ramus lateralis accessorius and the smaller one a dorso-spinal nerve. The section also passes through one of the depressor muscles. The medial and dorsal part of the section passes through the skin, which connects the basal part of the rays (the proximal parts of the rays not being included in the sectional plane). The epidermis of this fold of skin is similar in structure to that of the groove. Within the epidermis is a pigmented layer, and internally to the latter is loose connective tissue containing the horny fibres of the rays cut transversely, nerves, and blood-vessels. At the base of this fold of skin the head of the ray-carrier is seen,

with embryonal cartilage disposed dorsally to it, and then the articulating sphere for the ray.

The occurrence of numerous terminal or taste-buds ("becherförmigen Organe") in the epidermis of the groove bordering the fin is particularly noteworthy in connection with this investigation. These taste-buds, which project slightly on the surface of the epidermis, are bulb-like organs containing long, sensory cells. The taste-buds have a marginal layer of cells, which form a definite limiting membrane. The sensory cells consist of (1) a long, cylindrical, apical part terminating in a bristle which projects slightly on the surface of the epidermis, (2) an expanded part containing the nucleus, and (3) a basal part, which is continuous into one or more fibres. The taste-buds are also in connection with nerve-fibres.

Bateson has described similar organs on the barbels, pelvic fins and palate of the same fish, and the taste-buds, which I now bring under notice, are similar in structure to those described by him from these other parts.

I have also compared these taste-buds with the "neuromasts" or organs of the lateral line in the rockling, and agree with Herrick in his contrast of gustatory buds and neuromasts. The "neuromasts" are, as a rule, sunk beneath the skin in canals, tubes or pits, while taste-buds are superficial, or may slightly project on the surface. The specific, sensory cells of the neuromasts only extend partly through the space limited by the sensory epithelium, and thus do not reach the internal, limiting membrane; on the other hand, in taste-buds the sensory cells extend from the external to the internal boundaries. The sensory cells of neuromasts frequently end in hairs, and are therefore sometimes termed "hair-cells," while those of taste-buds may terminate in bristles but not in hairs.

A further contrast, which I may now refer to, is the mode of innervation. The vibratile region of the rockling is innervated partly by the ramus lateralis accessorius, and in part by branches of the dorso-spinal nerves. The ramus lateralis accessorius is a recurrent branch of the facial nerve, and

belongs to the system known to comparative anatomists as the "communis" system. The ramus lateralis accessorius, which has no connection with the nerve of the lateral line, is paired and takes its origin in the lobus facialis of the myelencephalon. It runs backwards (sending off branches on its way to the barbels and pelvic fin), one on either side of the ligament, which supports the vibratile fin, and in the region of the latter its branches anastomose with the spinal nerves at their ganglia. From this anastomosis the nerve-fibres of the ramus lateralis accessorius are sent along with general cutaneous branches from the spinal ganglia to the skin of the region of the vibratile fin. The main trunks of the ramus lateralis accessorius continue to run in a posterior direction, and at the origin of the second or aboral dorsal fin they rise to a higher level, and those of right and left sides become more widely separated from one another. My results regarding the innervation of taste-buds of the rockling agree with those of Herrick in his investigation of the gustatory organ of *Ameiurus*. According to Herrick, all "terminal buds" are innervated from a bilobed centre, namely, the gustatory tract or "visceral sensory column" of the brain, those of the mouth being connected with the posterior or vagal lobe by the vagus and glosso-pharyngeal nerves, and those of the skin with the facial lobe by means of the facial nerve.

I may at this point note the contrast that while the "neuromasts" or organs of the lateral line are innervated by the acustico-lateralis nerve taking its origin from the tuberculum acusticum or "somatic sensory column" of the myelencephalon, the terminal buds receive their nerve supply from the "communis" nerves arising from the vagal and facial lobes or visceral sensory column.

One may differentiate three systems of nerve-endings in the skin of fishes, namely: (1) The general, cutaneous nerve-endings innervated from the dorso-spinal roots; (2) the nerve-endings in the "neuromasts" innervated by the acustico-lateralis nerves, and (3) the nerve terminals of taste-buds supplied by the "communis" system of nerves.

My experiments to test the physiological value of the taste-buds consisted in bringing various forms of food into contact with, or into the proximity of the groove surrounding the vibratile fin. I find that it is of the utmost importance in these experiments that the rocklings should, firstly, have become thoroughly habituated to the artificial environment of an aquarium. The rocklings are so shy and easily disturbed that when brought in from the shore they are for some time too excited to have any desire for food.

The most successful experiments were made with a fish which had been many months in an aquarium, although with other rocklings which had been a week in captive conditions, similar, though not such satisfactory, results were obtained. A further item to be considered in these experiments is the rockling's hatred of light and of light-coloured surfaces. As the rockling is primarily a night-feeder, I conducted many of my experiments at night, and as showing their sensitiveness to light, it may be remarked that it was found necessary to keep the candle-light in a more or less shaded position. In spite of these hindrances, however, the reflexes or responses obtained were definite and precise.

In my earlier experiments I tried the effects of various extracts of beef. In these I directed a current of fluid beef extract by means of a pipette against the surface of the groove on which the taste-buds are situated. The result of this was a response on the part of the rockling accompanied by a swallowing of the liquid food. As a control experiment I directed a current of sea-water against the same parts, but was unable to detect any response.

Subsequently I tried similar experiments with small pieces of liver, the muscles of crayfish and fish, etc., which were held near the taste-buds by means of a thin wire; the result was that responses were obtained, but not so definite as might be desired. The clearest and most reliable reflexes or reactions were, however, obtained on using living lobworms as the bait. Small, living lobworms or parts of these were gently let down through the water upon the skin of the



groove surrounding the fin (the eyes of the rockling being at the same time concealed); a reaction was at once obtained, in which the fish either turned sharply round or rapidly "backed water" and seized the prey. This experiment was repeated again and again, and at intervals by day and night, and the response observed was always clear and definite. The effect of placing the food on the taste-buds was so evident as to be at the same time entertaining. If one lowers the bait until it is in contact with the taste-buds, and then very rapidly withdraws it, the fish responds, and then apparently loses the power of locating the food. In this connection it is interesting to notice that an observer states that one species of rockling rubs or rolls itself about its prey.

In experiments of this nature one must be careful not to ascribe to the physiological action of the taste-buds, reflexes or reactions which might be due to the action of the other sense-organs. As regards the sense of sight, the fish did not in my experiments locate the bait by this means, as in many cases the eyes were covered or concealed.

Regarding the sense of smell, it appeared to me that the reflexes were obtained before the odour of the bait had time to reach the nostrils. In regard to this point one may also refer to the work of Bateson and Herrick. Bateson in the section of his paper which treats of the taste-buds of the pelvic fins and barbels of the rockling, says that the fact that the rockling in which the olfactory organs had been removed, did not pay any attention to food that was not put close to it, tends to show that the taste-buds are of use only in actual contact with the food. Herrick holds that the taste-organs are more efficient than Bateson has supposed, and that the latter author did not sufficiently distinguish between the senses of taste and smell. He holds that Bateson's experiments were insufficient to demonstrate the real efficacy of the taste-buds. Herrick obtained reflexes from a tomcod, *Microgadus tomcod*, in which the olfactory organs had been extirpated; further, by letting down beef extract, which had been previously stained in order that

the diffusion currents might be observed, upon the taste-buds of the barbules of *Ameiurus*, the gustatory reflexes were obtained before the diffusing currents, as marked out by the stained fluid, reached the nostrils.

In regard to the sense of touch, in my experiments it seemed that the best-defined reflexes are obtained when the bait is placed in actual contact with the skin of the area under discussion, and this reaction may be regarded as a gustatory and co-related tactile response. One also, however, obtains well-defined reactions when the food is not actually in contact, but only in the proximity of the taste-buds, and this type of response may be regarded as a purely gustatory reflex.

In experiments, which consisted in using small pieces of cotton-wool instead of morsels of food, I also obtained tactile reflexes in which the fish on the first occasions seized the wool. It appears, however, that contact with cotton-wool is not sufficient to maintain the reflex for any length of time, and that the respective reflexes of taste and touch "can be experimentally isolated by training." As the result of my experiments I am inclined to agree with Herrick, who writes: "The final result seems to be that while the tactile sensation is not sufficient alone to maintain the reflex, the addition of the gustatory element is sufficient, and therefore that the gustatory element is the essential element in setting off the reflex." In an addendum to his valuable paper Herrick after further experimentation arrives at the conclusion with which I agree, that gustatory stimuli by themselves, and apart from the co-related tactile accompaniment, "can be localised in space or have a local sign"; although the response is not so strong and definite as the gustatory plus tactile reflex.

One may, therefore, with Herrick, distinguish four reactions: (1) A vague, seeking reaction, excited by the sense of smell, and consisting in an aimless, circling movement; (2) a quick and definite reaction, consisting in a sharp turn of the body and rapid seizing of the bait, which is obtained

when the food is placed in actual contact and is due to the co-related reflexes of touch and taste; (3) a reaction similar to the last, but not so definite, and which is observed when the food is not actually in contact, but only in the proximity of the taste-buds: this may be regarded as a purely gustatory response; (4) a tactile reaction, to which the fish at first responds, but after repeated experiments and then an interval only reacts in a tentative or inquiring manner, with a deliberate movement.

The main purpose of this paper is to indicate that the vibratile fin of the rockling is morphologically, as indicated by Bogoljubsky, a modified part of the ordinary dorsal fin, and physiologically a part which, together with the adjacent skin, forms a highly efficient food-locating or food-detecting organ.

A general correspondence in structure allows us to deduce that this oral fin is morphologically a modification of the aboral fin. The anterior rays of the aboral agree with those of the oral fin in general structure, and the two fins are directly connected with one another by a ligament, although there is a slight external interval between them.

As regards the physiological side, it has already been noted that the vibration of the rays keeps the skin on either side, on which the taste-buds are situated, clear of sand particles, etc. One has also to remember that internal taste-buds are usually associated, as in mouth, pharynx, gill-chambers, etc., with a current of water. I would also suggest that as the rockling is phlegmatic in its habits, and lives on the shore under stones in more or less still water during at least half its lifetime, or on the bottom in deeper water, the advantage of vibrating processes driving currents of water is obvious; this vibration no doubt aids in bringing indications of food. The experiments of Herrick with other fish, showing that these detected the presence of food by means of the taste-buds more quickly in running than in still water, is of interest in this connection.

As regards the belief held by some zoologists that the

vibratile fin is a "lure" enticing prey to destruction, I may point out that, apart from the fin's position some distance posterior to the month, this would be impossible in certain cases, as the prey either does not possess the power of sight or has only feeble visual power. In other cases it is probable that this part does excite curiosity and arrest attention, and that, by this means, the prey is brought within the sphere of action of the taste-buds. On the other hand, it is evident that as the rocklings lie more or less hidden in the sand, animals may come quite accidentally into the proximity of the terminal buds.

It is not suggested in this paper that external taste-buds are exceptional in fishes, but it is held that the vibratile fin region of the rockling is a localised and specially efficient taste- or food-locating organ.

In terrestrial animals the taste-buds are confined to the lips and month cavity, and in this case their function is rather to test than to search for food. On the other hand, the external taste-buds of fishes can be used in locating food, and complex reflexes are associated with this in order to effect the capture of food.

This work has been carried out at the Marine Stations of Millport and Cullercoats, and to the authorities at these institutions I must express my cordial indebtedness. I must also thank Dr. E. J. Allen, of the Marine Biological Station, Plymouth, who kindly sent me some material, and also my colleague, Mr. E. W. Shann, for collecting further specimens while working at Port Erin. To Mr. Walter H. Young, Cullercoats, my thanks are also due for some excellent photographs taken during the progress of my work.

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## EXPLANATION OF PLATE 11,

Illustrating Dr. J. Stuart Thomson's paper on "The Dorsal Vibratile Fin of the Rockling (*Motella*)."

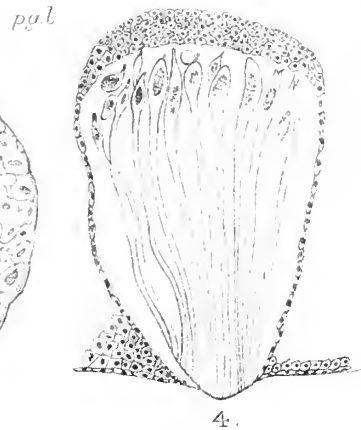
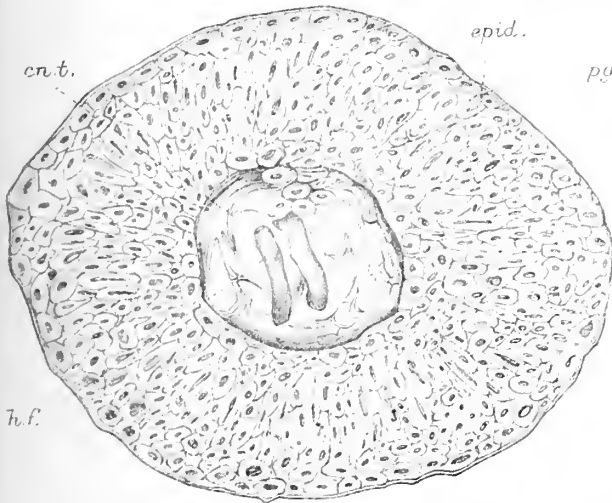
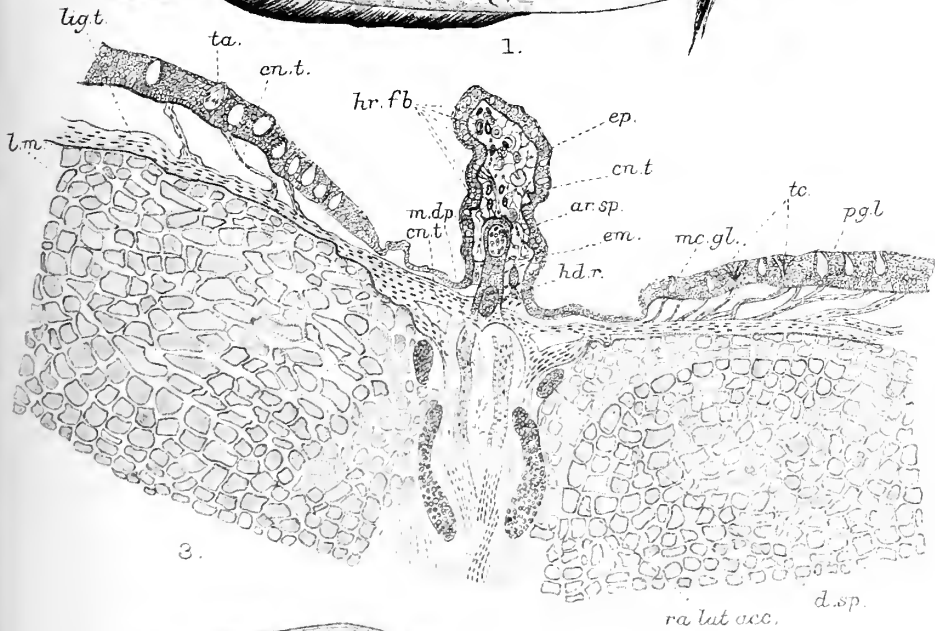
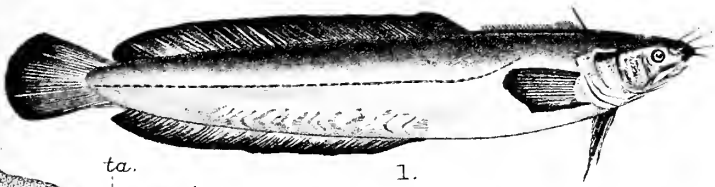
Fig. 1.—Lateral view of *Motella* showing the dorsal vibratile fin.

Fig. 2.—Dorsal view of *Motella* showing the vibratile fin.

Fig. 3.—Vertical section of the vibratile fin and adjoining parts. The section passes through the fold of skin which connects the individual rays basally, the upper parts of the rays not being in the plane of section. *ar. sp.* Articulating sphere for the rays. *cn. t.* Connective tissue. *d. sp.* Dorso-spinal nerve branch. *ep.* Epidermis. *em.* Embryonal cartilage. *hd. r.* Head of ray-carrier. *hr. fb.* Horny fibres of the rays. *lig. t.* Ligamentous tissue. *l. m.* Lateral myomeres. *mc. gl.* Mucous glands. *m. dp.* Depressor muscle. *pg. l.* Pigmented layer. *ra. lat. acc.* Ramus lateralis accessorius. *tc.* Tactile nerve-endings. *ta.* Taste-buds.

Fig. 4.—Terminal or taste-bud showing the sensory cells and the limiting membrane.

Fig. 5.—Transverse section through a ray. *ep.* Epidermis. *pg. l.* Pigmented layer. *cn. t.* Connective tissue. *h. f.* Horny fibres.







## Two New Species of the Phoronidea from Vancouver Island.

By

**H. L. M. Pixell, B.Sc., F.Z.S.,**

Demonstrator of Zoology and Reid Fellow, Bedford College,  
University of London.

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With 16 Text-figures.

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6 THE animals described in this paper were obtained while I was working at the Marine Biological Station, Departure Bay, Vancouver Island, in the summer of 1911 during my tenure of the Reid Fellowship. They include two species—one belonging to the genus *Phoronis* (Wright, 1856), the other to the genus *Phoronopsis* (Gilchrist, 1907).

### I. *PHORONIS VANCOUVERENSIS* N. SP.

This is a colonial form occurring in large compact, more or less hemispherical masses attached to the cretaceous sandstone forming the islands situated in Departure Bay. The colonies (Fig. 1) measure 5 cm. or more in diameter, and generally adhere to overhanging rocks near low-water mark. Each colony is composed of numerous individuals with brownish chitinous tubes, so very much intertwined that it is difficult to obtain a complete specimen from the tangled mass. The proximal ends of the tubes are rounded off, and the whole tube seems scarcely as long as the expanded animal. The total length of an average large specimen is 40 mm., the tentacles forming 2 to 3 mm. of this; the width

of a large specimen just below the lophophore is rather less than 1 mm., while the ampulla measures 1.2 mm. in diameter.

The animals are colourless and transparent except for a greater or smaller number of irregularly arranged opaque white spots. These spots are quite conspicuous in the living

TEXT-FIG. 1.



Part of a colony of *Phoronis vancouverensis* from a photograph. ( $\times 2$ .)

animals, and are caused by masses of minute granules on the surface of the epidermis, occurring chiefly on the tentacles and distal region of the body. They can be scraped off the surface of the animal, and in cutting sections the granules become liberated, and obscure to a considerable extent the cell details in the animals, in which they are numerous. The masses are white and opaque when viewed with reflected light, but the individual granules are more or less transparent and refringent with transmitted light. The

nature of this pigment will be considered later in relation to the vaso-peritoneal tissue.

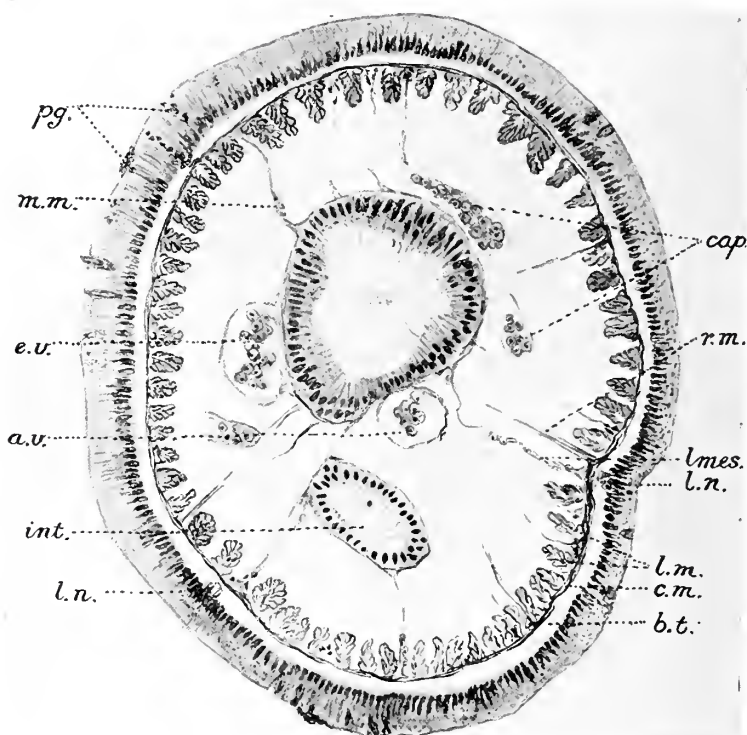
The lophophore is somewhat horseshoe-shaped, and is provided with about ninety tentacles; these varied in specimens counted from about 72 to over 100, but the average for ten fairly typical forms was 90.9.

The lophophore organ is absent—at least I have found no trace of it in the series of specimens (twenty-five to thirty) that I have examined; this is probably due to the fact that the specimens were collected during the beginning of September, when practically all the generative products had been shed, and a brood chamber, as Gilchrist (7) supposes this organ to be, would no longer be required.

Body-wall.—The epithelium is composed of tall columnar cells, except over the greater part of the ampulla, where they are almost cubical (Figs. 2, 3, 4).

The glandular cells are numerous, and have three different contents: (i) very fine granules, (ii) much coarser spherical granules or globules, 2 to 3  $\mu$  in diameter, and (iii) a homogeneous mucus, which may sometimes be seen protruding on to the surface, leaving the goblet cell below empty (Fig. 12, *m. g.*). All these contents are yellowish and refringent when unstained; they do not stain easily except with iron-haematoxylin. They are all found in the upper regions of the body as well as on the ampulla, in this resembling, according to Selys Longchamps ([14], p. 38), *Ph. psammophila* and *Ph. sabatieri* rather than *Ph. hippo-crepia*.

The proximal region of the ampulla is covered with very long columnar cells, interspersed with numerous glandular ones, whereas *Ph. australis* has no glandular cells, or very few in this position (Benham [2], p. 11). These long epidermal cells extend for a short distance up the sides, and gradually merge into the typical cubical epithelium covering the ampulla. The proximal body-wall is often invaginated to form a pit, but this is by no means always the case, and in one that had a convex, proximal end I found in longitudinal

TEXT-FIG. 2.<sup>1</sup>

Transverse section through the lower œsophageal region to show low irregular fascicles of longitudinal muscles. Formula:

$$\frac{1922}{713} = 61. \quad (\times 180.) \quad \text{For lettering see footnote.}$$

<sup>1</sup> [Figs. 1 to 5 are of *Ph. vancouverensis*, and Figs. 6 to 16 of *Phoronopsis harmeri*.]

[The abbreviations used are the same throughout the paper.]

#### LETTERING OF THE FIGURES.

*a.c.* Anterior coelom. *a.p.* Anal papilla. *a.v.* Afferent vessel. *b.c.* Red blood-corpuscles. *b.t.* Basement tissue. *c.* Collar. *cap.* Capillary coeca. *c.b.* Ciliated band in pregastric region of digestive tube. *c.m.* Circular muscles. *col.ep.* Columnar epithelium. *cu.ep.* Cubical epithe-

sections that the mesentery which attaches this extremity to the bend in the digestive tube during life was broken; consequently it seems probable that the pit which has been so often described is only caused by tension in this mesentery due to its contraction. A similar slight concavity is frequently seen in the body-wall at the insertion of the lateral mesenteries (Figs. 2 and 4, *l. mes.*).

The basement tissue (Figs. 2 and 5, *b. t.*) consists of a homogeneous membrane without any cells such as have been observed in *Phoronopsis harmeri*, subsequently to be described.

**Muscular Layers.**—The circular muscles seem to be as usual in other species of *Phoronis*, and the distal region of the body is traversed by numerous radial muscles (Figs. 2 and 5, *r. m.*).

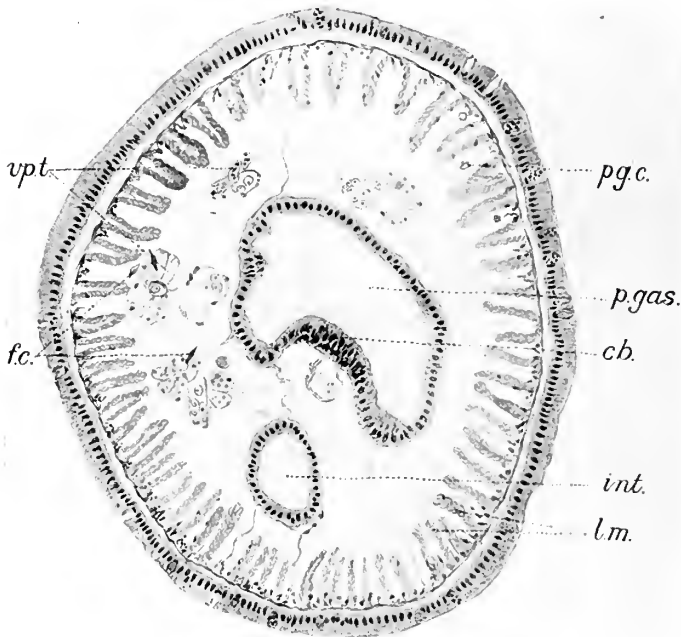
The longitudinal muscles are greatly developed, and differ from those of all species so far described in the fascicles having a different character at different levels. In the distal region the fibres are arranged somewhat irregularly to form fascicles as described for *Ph. hippocrepia*, *Ph. buskii* (McIntosh [12]), *Ph. australis* (Benham [2]) (Fig. 2, *l. m.*), whereas in the region of greatest development, i. e. about

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lium. *d.* Diaphragm. *d. a.* Digestive areas of stomach. *d. b. c.* De-generating blood-corpuscles. *ep.* Epistome. *e. v.* Efferent vessel. *f.* Nerve-fibrils. *f. c.* Fusiform corpuscles. *fol.* Follicle cells of ova. *g.* Ganglionic mass. *g. g.* Epithelial glands with spherical granules. *gr.* Groove in stomach. *gr. p.* Granular peritoneum. *h. p.* Hypertrophied peritoneum round afferent vessel. *int.* Intestine. *i. t.* Tentacle of inner series. *l.* Lumen of afferent vessel. *l. m.* Longitudinal muscle. *l. mes.* Lateral mesentery. *l. n.* Lateral nerve. *l. o.* Lophophoral organ. *m. g.* Mucous glands. *m. mes.* Median mesentery. *n.* Nuclei. *n. d.* Nephridial duct. *n. d.* Terminal part of nephridial duct. *n. f.* Nephri-dial funnel. *n. p.* Nephridiopore. *n. r.* Nerve-ring. *n. t.* Nervous tissue. *o.* Ova. *œs.* Œsophagus. *o. g.* Oil-globules. *o. t.* Tentacle of outer series. *p.* Peritoneum. *p. c.* Posterior cœlom. *p. g.* Pigment-granules. *p. gas.* Pregastric region of the digestive tube. *pg. c.* Pigment-bear-bearing corpuscles. *pg. s.* Pregastric sinus. *r.* Rectum. *r. m.* Radial muscles. *st.* Stomach. *vp. t.* Vaso-peritoneal tissue. *y.* Yolk-spherules.

one third from oral end, they have the pinnate arrangement found in *Ph. psammophila* (Cori [3]), *architecta* (Andrews [1]), *pacifica* (Torrey [15]), i. e. the fascicles appear feather-like in transverse section.

TEXT-FIG. 3.



Transverse section through the pregastric region to show the high pinnate character of the fascicles of longitudinal muscles. ( $\times 150$ .)

Frequently there are 19 of these in the left anterior cavity, 22 in the right anterior, 13 in the right posterior and 7 in the left posterior, or according to Selys Longchamps' convenient formula—

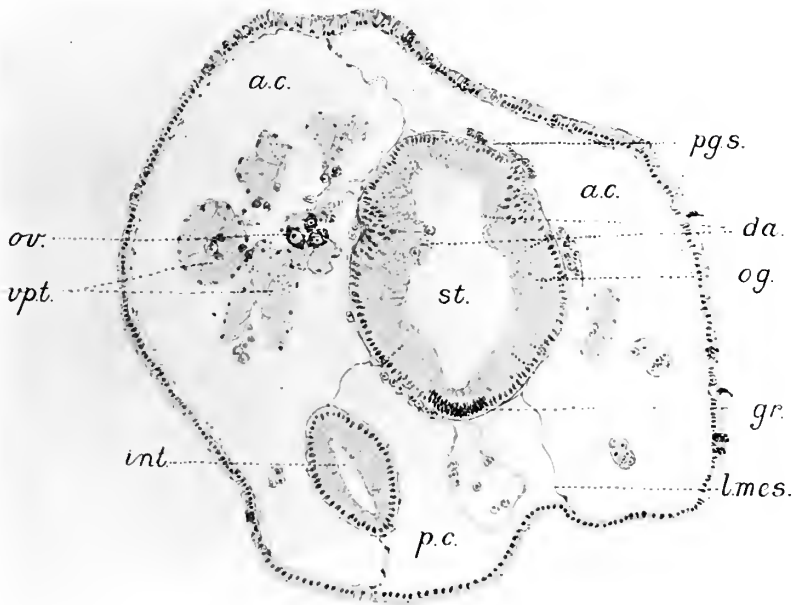
$$\frac{19 + 22}{7 + 13} = 61 \text{ as in fig. 2.}$$



Or there may be more in the anterior cavities as in Fig. 3 ;

$$\frac{24}{4} \bigg| \frac{24}{7} = 59.$$

TEXT-FIG. 4.



Transverse section through ampulla showing coelom divided into two anterior cavities (*a.c.*) and one posterior cavity (*p.c.*). ( $\times 150$ .)

Shortly behind this region the posterior coelom appears to be undivided, or rather the left post-coelom merges with the anterior owing to the disappearance (Fig. 4) of the left lateral mesentery.

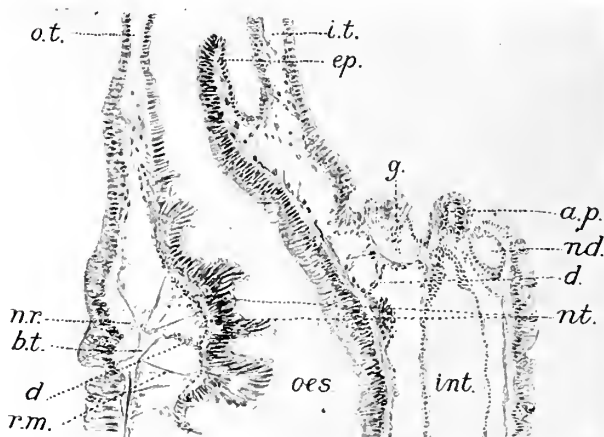
*Ph. hippocrepia*, which in many ways closely resembles *Ph. vancouverensis*, shows in my sections only about twenty-eight fascicles of longitudinal muscles. Selys Long-

champs states that in this species they do appear to vary particularly, but gives as the highest recorded by anyone :

$$\frac{12}{6} \bigg| \frac{13}{7} = 38.$$

This is a great deal lower than the average number of sixty in *Ph. vancouverensis*, and such an anatomical characteristic seems to be of far more importance from a systematic

TEXT-FIG. 5.



Longitudinal section through the base of the lophophore approximately in the median dorsal lines. ( $\times 100$ .)

point of view than such variable details as size and number of tentacles. Both Cori (3) and Selys Longchamps (14) point out that within limits the number of fascicles of longitudinal muscles is constant.

The diaphragm or transverse septum (Fig. 5, *d*) slopes upwards from the oral to anal side, meeting the dorsal surface just in front of the anal papilla. The two layers of peritoneum covering it are widely separated at the sides by the basement membrane, which is continuous with that of the body-wall near the lower border of the nerve-ring, but towards its centre this median layer of the septum is so thin

that it may easily be passed over. I do not think, however, that it is entirely eliminated as in *Ph. capensis* described by Gilchrist (7), who suggests that such a character of the septum would probably be of value in specific determination, stating that *Ph. hippocrepia* agrees with *Ph. australis* in having the septum uniformly invaded by a basement tissue. In *Ph. vancouverensis* there is certainly no uniform median layer, so I have included this characteristic in the table at the end as a minor feature distinguishing this specimen from *Ph. hippocrepia*.

The mesenteries also contain a very thin layer of basement tissue.

**Nervous System.**—The ring of nervous tissue at the base of the lophophore is quite apparent (Fig. 5, *n. r.*) and is continued posteriorly, following the course of the tentacles, up each of which passes a fine strand of the same tissue. Across the dorsal surface in front of the anus is a large ganglionic mass (Fig. 5, *g.*) composed of the usual punctated tissue with definite striation and numerous cells with large nuclei. This tissue is everywhere in close relation with the inner ends of the elongated epithelial cells.

In some sections can be seen a small lateral nerve-cord running along each side of the body close to the point of attachment of the lateral mesenteries (Fig. 2, *l. n.*), and projecting into the basement tissue as a small mass of punctated tissue. These appear to be very short, for they have not been seen beyond the œsophageal region.

Traces of nervous tissue have been observed in the centre of the pit at the proximal end of the body and also along the alimentary canal on the outer side of the epithelium. This is especially marked in the upper œsophageal region opposite the nerve-ring. Gilchrist (7) mentions this sensory patch and suggests that it may represent an organ of taste.

**Alimentary Canal.**—The various regions of the alimentary canal are named in the figures in accordance with Cori's views (3), these being also adopted by Selys Longchamps (14).

The œsophagus (*œs.*), with its thick walls often much folded, only extends for a short distance. Fig. 2 is a transverse section through its posterior part where the two branches of the lateral vessel have just united.

This is followed by the pregastric region (Fig. 3, *p. gas.*) ("préestomac" of Selys Longchamps, Vormagen of Cori) which has a large cavity irregular in shape with thin walls. The epithelium is cubical, ciliated, and has oval nuclei. Along the postero-median region close to the afferent vessel is a longitudinal band of epithelium (Fig. 3, *c. b.*) thicker than the rest and with several layers of elongated nuclei. This has been described in *P. pacifica* (Torrey [15]) and in *P. architecta* (Andrews [1]), but is very little developed in *Ph. hippocrepia*.

The stomach (Fig. 4, *st.*) is the large terminal region of the descending part of the tract situated in the ampulla. It has a thick epithelium with ovoid nuclei. On the postero-median side there is a longitudinal groove directly continuous with the thickened band in the pregastric region and having similarly several layers of nuclei which stain more deeply than the ordinary ones (Fig. 4, *gr.*). The cells along this groove have very long cilia. The digestive areas which carry on intra-cellular digestion (Fig. 4, *d. a.*) have no cilia, and the free ends of the cells are distinctly amœboid during functional activity. The whole of the ascending limb of the alimentary canal is called the intestine except for the very short part contained in the anal papilla, which Cori considers to be a proctodæum and calls the rectum. The part of the intestine in the ampulla (Fig. 4, *int.*), the only part called "intestine" by Benham, has thick, closely ciliated walls and generally an oval lumen. The epithelium is cylindrical, with oval nuclei. The upper part of the intestine (figs. 2 and 3, *int.*), has cubical epithelium, with long cilia and large nuclei. In transverse sections the intestine appears oval or elongated, only occasionally showing the usual triangular shape due to the pull of the three mesenteries.

The cells in the lower part of the stomach and intestine

are frequently seen to contain rows of spherical deeply-staining masses, probably oil-globules (Fig. 4, *o. g.*), such as are often seen in the intestinal cells of mammals. They appear to collect near the outer borders of the cells and to be taken up by the blood-corpuscles in the surrounding sinus. Some blood-corpuscles appear to be crowded with these globules, which appear slightly smaller than the nucleus—possibly after slight chemical changes this product is deposited in the vaso-peritoneal cells as yolk-spherules.

One specimen contained a few nearly spherical coccidia about  $50\ \mu$  in diameter in some of its intestinal cells; these are the only parasites that I have observed in either of the species.

**Vascular System.**—The general arrangement of the blood-vessels can be made out without difficulty in the living animals, for owing to the red colour of the blood it can easily be seen through the transparent body-wall. The two longitudinal vessels, the lateral or efferent and the median or afferent, extend the whole length of the alimentary canal, and at intervals only near the proximal end connect with the perigastric sinus by the breaking down of the intervening walls (Fig. 4). I have seen the median vessel very dilated and crowded with corpuscles quite close to the bend in the digestive tube.

The numerous capillary cœca project freely into the cœlom, and do not branch as they do in *Ph. australis* (Benham [2], fig. 18) and in *Phoronopsis harmeri*, to be described later. The corpuscles are  $8\text{--}10\ \mu$  in diameter, and have a pale yellow colour when seen singly. They take up eosin easily, and the nucleus and granules stain readily with iron-hæmatoxylin, but I have not found them to stain well with either Delafield's hæmatoxylin or carmine.

In some specimens the corpuscles in various stages of development may be seen along the afferent vessels, being developed from the lining epithelium (Fig. 15, *b. c.*) just as shown by Cori (3, pl. xxvii, figs. 2 and 3).

A fine granular precipitate is present in some of the

vessels, indicating the presence of a serum as well as corpuscles.

**Excretory System.**—There are as usual a pair of excretory organs at the distal end; they are small tubes bent once only on themselves, and each opens into the coelom on either side of a lateral mesentery by a small funnel. That into the posterior coelom has a process extending down the mesentery for a distance of about  $160\ \mu$ ; they are both closely applied to the transverse septum above.

The tube, which is about  $250\ \mu$  long, runs first outwards close to the mesentery, and then upwards embedded in the basement tissue, forming a slight ridge visible on the outside; it then turns inwards, running along the dorsal surface to open on one side and in front of the anus.

The question as to how far the general peritoneum and the blood-corpuscles derived from it have retained their excretory function is considered later.

The Vasoperitoneal tissue (Gefässperitonealgewebe of Cori [3] or nutriment tissue of Ikeda [9], and constituting Kowalevsky's corps adipeux) is developed on some of the capillaries on both sides of the digestive tube (Figs. 3, 4, *vp. t.*). It consists of the usual large flat cells with small nuclei at their outer ends. The contained yolk-spherules vary much in size and stain easily with eosin, Licht grün and other stains. Iron-hæmatoxylin can be washed out of them more easily than from the nuclei.

Blood-corpuscles apparently in various stages of degeneration are also to be seen in the cells (Fig. 16, *d. b. c.*), and generally some fusiform corpuscles (*f. c.*). These are often especially numerous in the pre-ampulla where the vasoperitoneal tissue is only present in small amount, and they have also been observed floating freely in the coelomic fluid, occasionally in the distal end right away from this tissue. Ikeda (9, p. 145) states that he has never found these corpuscles anywhere in *Ph. ijimai*, and thinks they are of no great physiological importance. I have sometimes found them in immense numbers, giving no sign of a nucleus, but generally showing

a delicate striation. They do not stain with Delafield's hæmatoxylin, but take up iron-hæmatoxylin and stain homogeneously like the yolk-spherules.

Besides the above substance quantities of refringent non-staining granular substance (Fig. 16, *p. g.*) occur in the vaso-peritoneal tissue, either in separate granules or massed together, and contained in corpuscles similar to the sphæruleferous corpuscles described by Durham (4, p. 329) in *Asterias rubens*.

I have seen corpuscles associated with degenerating blood-corpuscles or with fusiform bodies and others filled to a greater or less extent with these refringent granules, in the vaso-peritoneal tissue (Fig. 16, *pg. c.*), and also free in the body-cavity, sometimes close to the body-wall between the fascicles of longitudinal muscles (Figs. 3 and 14, *pg. c.*).

It is apparently these same bodies which are often to be seen traversing the body-wall (Fig. 12, *pg. c.*), and they seem to be similar to the wandering cells, described by Durham in Echinoderms (5, p. 88), which are able to get rid of effete material from the system. The granules are set free on the surface (Figs. 2, 12, *p. g.*), where they form the opaque white pigment masses so conspicuous in some specimens on the outside, especially on the tentacles and distal parts of the body.

Micro-chemical tests showed that these granules are unaffected by weak acids, alkalies or ether, as well as by the ordinary reagents and stains. They also give the murexide reaction, and therefore contain some uric acid compound. It seems probable that this may be guanin, which is such a common excretory substance in many invertebrates.

The deposit of these pigment granules in the distal regions of the body may be accounted for by the action of light, but, if, as I feel confident, they are excretory products, we should expect, as Harmer (8, p. 122) has pointed out for the excretory vesicles of *Tubulipora*, that they would occur chiefly in such regions where waste products would be most easily carried away.



I cannot be sure whence these pigment-bearing corpuscles arise. They may be derived from the peritoneum, which is so greatly hypertrophied in places, e.g. on the afferent vessel (Fig. 15, *h. p.*), where the cells enlarge and then appear to be liberated, also probably some of the cells covering the capillary cœca develop into such corpuscles instead of ordinary vaso-peritoneal tissue. Cells having such an origin would be in close relation with the blood-corpuscles, and hence able to extract excretory substances similarly to the chloragogen cells of *Oligochætes*.

Eisig states that in the Capitellidæ (6, p. 758), whose nephridia are limited to small regions of the body, certain peritoneal cells laden with concretions are liberated into the body cavity (p. 762), and that both blood and peritoneum play an important part as true excretory organs, and not merely as conveyors of excretory products to other organs.

The subject of the excretory pigment in *Phoronis* seems to require further study. I have never seen any such pigment in *Ph. hippocrepia*, which is the only other species of *Phoronis* that I have personally examined, and can find no reference to any. Gilechrist (7, p. 154) mentions the presence of white pigment-spots irregularly arranged on the tentacles of *Phoronopsis albomaculata*, but he states that these consist of finely branching chromatophores.

Generative Tissue.—All the specimens examined for reproductive cells contained ova only, and of these only a few on the left side of the animal arising from the walls of capillaries close to their origin from the efferent vessel (Fig. 4, *ov.*). It seems probable that this species is protandrous, or possibly dioecious.

Affinities.—In size and mode of growth this *Phoronis* resembles somewhat closely *Ph. iijimai*, whose external characters were briefly described by Oka (13, pp. 147–8), and which was separated from other species owing to difference in the length or number of tentacles. Ikeda, who has studied the structure of *Ph. iijimai* (9 and 10) and has compared specimens of *Ph. hippocrepia* with it, says that

he is unable to discover any points by which they can be differentially diagnosed, pointing out (9, p. 582) that the length and number of the tentacles vary tremendously from one season of the year to another.

I therefore assume that both *Ph. ijimai* and *Ph. kowalevski* are encrusting varieties of *Ph. hippocrepia*, and from a comparison of specimens and sections of the latter and from descriptions given by other writers I conclude that there are several important anatomical differences separating it from *Ph. vanconverensis*. In the absence of any definitely formulated features by which the various forms included in the genus *Phoronis* may be separated, I think that such anatomical characteristics must be far more important from a systematic point of view than such variable details as size and number of tentacles.

Characteristics of *Phoronis vanconverensis*, which it is suggested should distinguish it from *Ph. hippocrepia* and its varieties:

- (1) The character and greater development of the fascicles of longitudinal muscles.
- (2) The presence of two nerve-cords in the anterior region of the body.
- (3) The structure of the diaphragm.
- (4) The well-developed band of specialised cells in the pregastric region.
- (5) The possible separation of the sexes (dioecious), or, if monœcious, then protandrous.

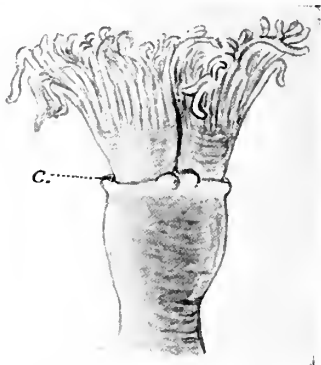
## II. PHORONOPSIS HARMERI N. SP.

This animal is placed in the genus *Phoronopsis*, established by Gilchrist (7) to include the form *Phoronopsis albo-maculata* described by him from South Africa, on account of the following characteristics:

- (1) The nerve-ring lies in an involution of the epidermis.
- (2) Only the left nerve-cord is developed.
- (3) The longitudinal muscles of the body are in numerous well-developed fascicles.

Habitat.—Specimens of the animal are easily obtained from the sandy shores of some of the smaller islands in the neighbourhood of Nanaimo. The tubes, 100 to 150 mm. long with a diameter of 3 to 4 mm., are found embedded in a vertical position with their upper ends slightly below the surface of the sand near extreme low-water mark, their positions being only indicated by minute holes in the sand above. The tubes are cylindrical, composed of a hard resistant membrane coated with fine sand grains; the lower end is rounded

TEXT-FIG. 6.



Posterior view of distal region of *Phoronopsis harmeri*. ( $\times 10$ .)

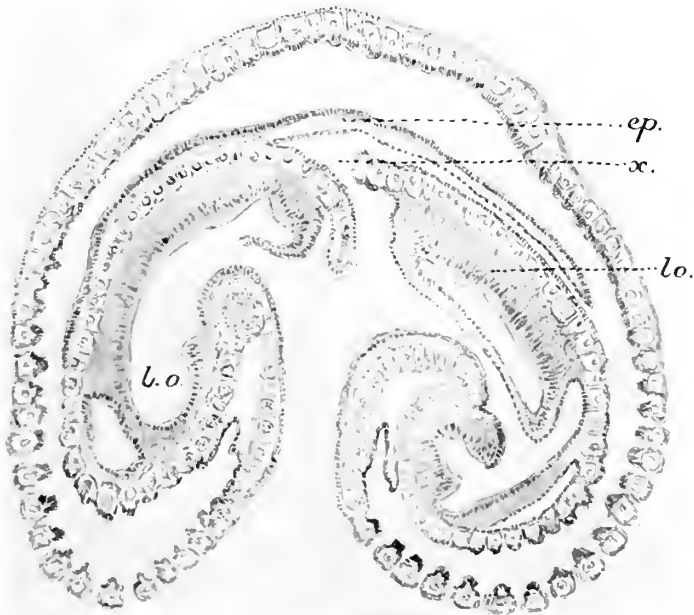
off, forming a blunt point. In some the distal end was limp and had no sand grains attached to it.

The animal so completely fills the circumference of the tube that it is only with difficulty removed.

Colour.—The tentacles and distal part of the body generally appeared during life to be of a pale greenish colour, and to be more or less covered with opaque white spots of exactly the same nature as in the case of *Ph. vancouverensis*. The ampulla was brownish-red. The last 5 or 6 mm. in some specimens were clearer and separated by an annular constriction. The red blood-vessels were clearly visible through the body-wall, and the rectum could often be recognised owing to its dark contents.

Size.—One of the largest specimens measures 147 mm. in length after preservation, 65 mm. of this being the ampulla, which, as a rule, seems to extend for nearly half the length of the animal. Some specimens are slightly under 100 mm.

TEXT-FIG. 7.



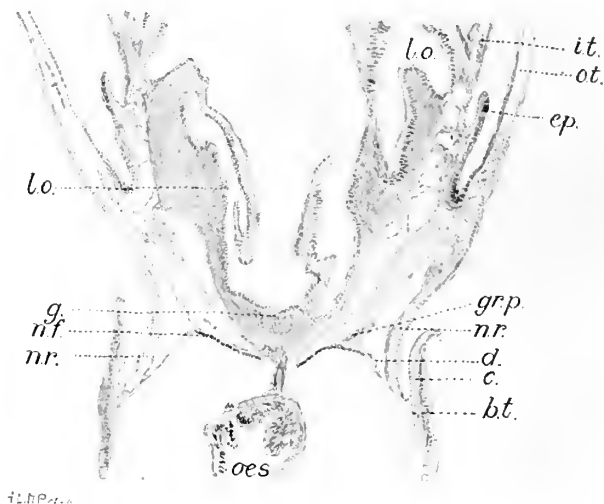
Transverse section through lophophore showing lophophoral organ (*l.o.*), and gap in inner row of tentacles (*x.*). ( $\times 50$ .)

long. The tentacles, which are frequently found regenerating, are, when fully grown, 3 to 4 mm. long (Fig. 6). The general width of the body is 1 to 2 mm. in the anterior region and 3 mm. across the ampulla.

Lophophore.—The extent to which the lophophore is coiled is shown in Fig. 7, from which it may be seen that there is a more distinct spiral than in the horse-shoe form of *Phoronopsis albomaculata*. The tentacles are also more numerous than in this species, for which Gilchrist

gives 126, being in every case over 200 (215 to 230). At the base of the tentacular membrane is the very distinct collar formed as a fold of the body-wall just behind the nerve-ring (Figs. 6, 8, 9, 10, *c.*). This collar is deeper at the sides than on the oral surface, and on the anal side it is interrupted by a bifid process, the anal papilla, into which the trunk cœlom is continued, and which is divided internally by the rectal mesentery suspending the short terminal rectum (Fig. 11).

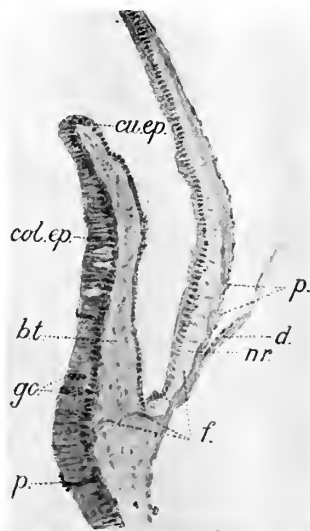
TEXT-FIG. 8.



Longitudinal section through the collar and base of lophophore  
in front of anal papilla. ( $\times 40$ .)

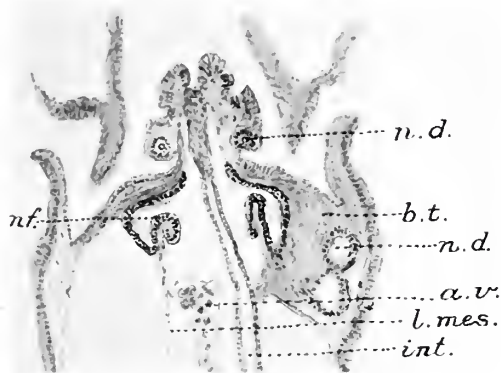
The lophophoral organ (Figs. 7 and 8 *l.o.*) is extremely variable; in some specimens it was large, and apparently similar to that described for *Ph. psammophila* by Cori (3) and for *Ph. capensis* by Gilchrist ([7] p. 158). The inner leaf-like fold forms a covered passage from close to the nephridiopores forwards and outwards into the lateral lophophoral spaces, which are lined by thick glandular epithelium. This epithelium also extends up the inner side of the tentacles, and has been seen by Gilchrist to secrete mucus by which the

TEXT-FIG. 9.



Part of a longitudinal section through collar region on one side.  
( $\times 100$ .)

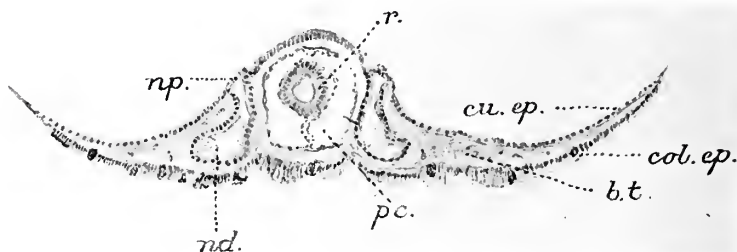
TEXT-FIG. 10.



Longitudinal section through anal papilla. ( $\times 50$ .)

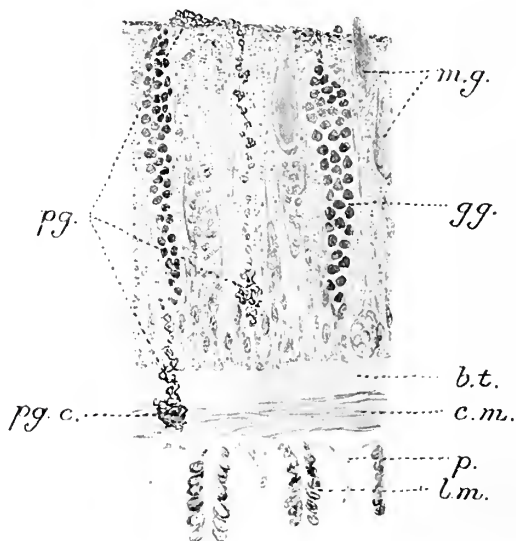
eggs are held together, and this author refers to the organ as a brood-chamber.

TEXT-FIG. 11.



Transverse section through collar and anal papilla at the level of the left nephridiopore. ( $\times 60$ .)

TEXT-FIG. 12.



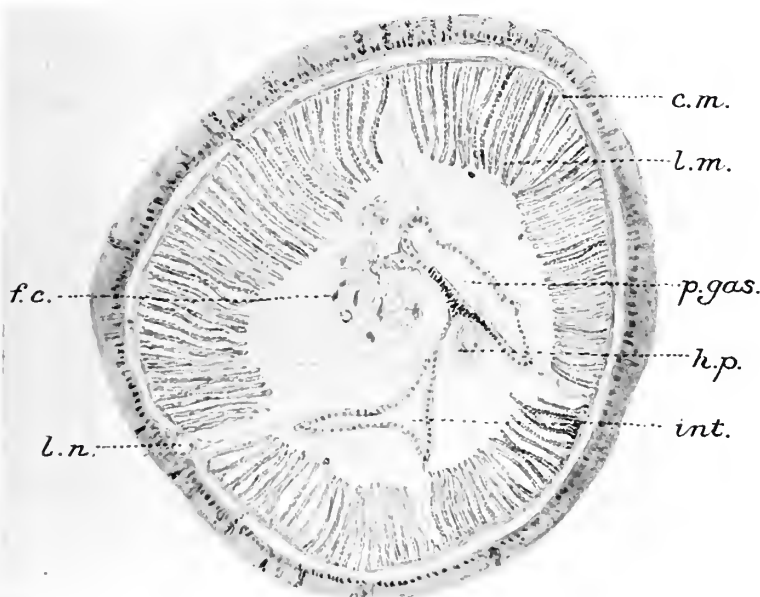
A portion of a transverse section of the body-wall towards the distal end; only the outer ends of the longitudinal muscles (*l. m.*) are shown. ( $\times 660$ .)

In the majority of specimens the organ is absent, which is



probably to be accounted for as in *Ph. vancouverensis* by the lateness of the season. The ovaries are in *Phoronopsis* more full of ova, though both were collected in September. Specimens with a lophophoral organ can easily be distinguished by the greater width of the tentacular crown.

TEXT-FIG. 13.



Transverse section through the oesophageal region showing the high pinnate fascicles of longitudinal muscles. ( $\times 60$ .)

Body-wall.—The epidermis (Fig. 12) agrees with that described for *Ph. vancouverensis*, and the excretory pigment is here again very noticeable. The same remarks apply to it as given for the former species.

The basement tissue (Figs. 9, 10, 11, *b.t.*) has here numerous small cells embedded in the clear matrix similar to that described by Benham (2) for *Ph. australis*.

**Muscular Layers.**—The circular muscles are as usual (Fig. 13, *c.m.*), and a few radial muscles traverse the coelom, especially in the distal part of the body.

The longitudinal muscles (Fig. 13, *l.m.*) are very greatly developed. The fascicles are more numerous than in any other described species of the Phoronidea. The usual number in the region of greatest development, i. e. about 50 mm. from the distal end, is about 126. These longitudinal muscles are distributed as follows: 41 in the left oral chamber, 42 in the right, 23 in the left anal chamber, and 20 in the right, or according to Longchamps' formula—

$$\frac{41 \mid 42}{23 \mid 20} = 126.$$

The fascicles are pinnate in transverse sections, and may extend inwards to a distance of 160  $\mu$ .

The nuclei of the peritoneum covering them are very prominent (Fig. 12, *p.*). Between the muscles a fold of the peritoneum extends inwards for a short distance; four of these folds are much longer, and reach the alimentary canal forming the mesenteries which divide the body cavity into the two anterior or oral and two posterior or anal compartments.

The collar (Figs. 5, 7, 8, 9, 10, *c.*) contains no coelom, but consists of basement tissue with numerous small cells. The epithelium covering its outer side is columnar and similar to that over the general body surface (*col. ep.*), but at the tip and down the inner side it is replaced by small cubical cells (*cu. ep.*). There are no muscles developed in connection with the collar, so that though it suggests the introvert of the Sipunculoidea and Polyzoa it is apparently quite functionless in that respect. Gilchrist (7) suggests that "it is the remnant of an ancestral introvert which has been retained with the new function of protection of the nerve-ring."

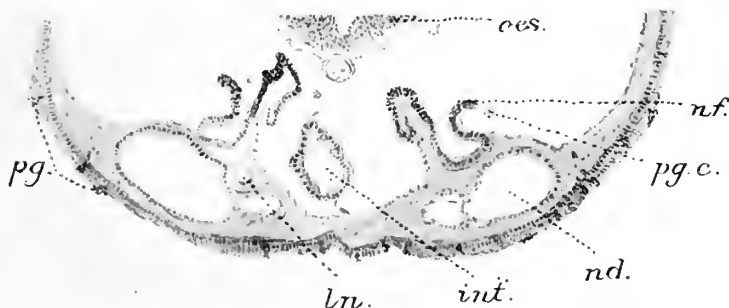
The diaphragm (Figs. 8, 9, *d.*) is thin, but apparently consists of the usual three layers. I have seen it appear to branch owing to the emergence of a blood-vessel, which has evidently been running for some distance obliquely across the region where the glandular layer of the nephridial funnel

is closely opposed to the septum. I do not know whether this would account for the septum appearing to give off an off-shoot towards the epistome, which Gilchrist (7, p. 156) stated required further examination.

Some of the peritoneal cells covering the part of this blood-vessel in the lophophoral cœlom near to the septum appear to hypertrophy, and become granular and possibly assume an excretory function.

Nervous System.—The nerve-ring has its usual position

TEXT-FIG. 14.



The posterior part of a transverse section through the region of the anterior nephridial funnels. ( $\times 60$ .)

about the level of the diaphragm, but is protected on its outer surface by the very distinct collar.

The punctated substance forming the ring (Fig. 9, *n. r.*) contains a few cells and is traversed by delicate fibrils. These, I think, must be nerve-fibres going to the epidermal cells, and not merely inner boundaries of these cells as has also been suggested, for similar small bundles of fibres pass out at intervals and cross the base of the collar and enter the epidermal cells, forming its outer layer (Fig. 9, *f.*).

The whole layer of nervous tissue forming the ring is narrower and more elongated than in most species of Phoronis, and there is no indication of its becoming separated from the epidermis, as Gilchrist (7, p. 156) suggested might possibly

occur in a completely developed specimen. On the anal side it turns inwards, following the curve of the lophophore, and is connected from side to side just in front of the anal papilla by a large ganglionic mass (Fig. 8, *g.*). From this passes downwards on the left side the conspicuous nerve-cord. In the nephridial region it is separated from the epithelium, and is embedded in the basement tissue (Fig. 14, *l. n.*). After passing internally to the nephridial duct (*n. d.*) it turns outwards and rejoins the epithelium a little to the oral side of the left lateral mesentery. From here it extends nearly to the ampulla as a very conspicuous cord in contact with the epithelium, but protruding slightly into the basement membrane (Fig. 13 *l. n.*). The centre is occupied by a clear substance which stains only slightly and around this are the nerve-cells.

**Alimentary Canal.**—In the stomach the ciliated groove with deeply chromatic nuclei is much smaller and less noticeable than in *Ph. vanconverensis*. The distal part of the intestine is markedly triangular in section and lined with small cubical cells (Fig. 13, *int.*), the short terminal rectum having columnar cells (Fig. 11, *r.*); the coelom is divided into its usual four compartments even in the ampulla as far as the bend in the digestive tube.

**Vascular System.**—The corpuscles are but very slightly larger than in *Ph. vancouverensis*, being on an average 10–12  $\mu$  in diameter, and the same remarks as to staining, etc., apply to them.

The coeca frequently branch, which they have never been seen to do in *Ph. vanconverensis*.

**Excretory System.**—The excretory tubes have the usual position, but differ slightly from those hitherto described. Each has a large funnel opening into the anterior coelom and a smaller one higher up opening into the posterior coelom; neither of these has its wall prolonged downwards for any distance as is usually the case. In addition to these funnels there is a wide orifice for communication between the anterior and posterior coelomic spaces owing to the lateral mesenteries

not meeting the œsophagus for some distance below the transverse septum.

The lower lip of the large anterior funnel wraps over the top of the lateral mesentery as shown in Fig. 10, *n.f.* It also extends round the sides and inner ends of this mesentery where it is free from the œsophagus (Fig. 14, *n.f.*). The top of the funnel is closely applied to the septum (Fig. 8, *n.f.*). Below the funnels the duct runs downwards for a short distance close to the mesentery in the anterior cœlom, then

TEXT-FIG. 15.



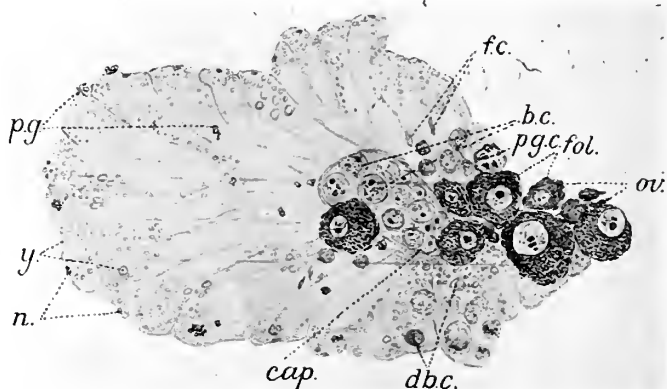
Transverse section through afferent vessel showing developing blood-corpuscles and hypertrophied peritoneum (*h.p.*) on the outside. ( $\times 660$ .)

turns outwards, and for the rest of its length is embedded in the basement tissue of the body-wall of the trunk. About .5 mm. below the septum it bends on itself. The ascending part of the tube is often much distended; it passes obliquely upwards externally to the nerve-cord on the left side (Fig. 14, *n. d.*), and on reaching the collar region narrows considerably and runs forwards embedded in the walls of the anal papilla to open a little in front of and below the anus (Fig. 11, *n. p.*).

The funnel consists of deeply staining ciliated epithelium and the duct is lined with ciliated cubical cells as usual.

The vaso-peritoneal tissue is developed on the walls of the capillaries on both sides of the body in the anterior cœlomic spaces, and the peritoneum covering the afferent vessel in the posterior cœlom is frequently greatly hypertrophied. These latter cells, however, appear to become detached, so that there is generally only one layer of them (Figs. 13 and 15, *h. p.*). They may possibly give rise to pigment-bearing corpuscles as suggested in the case of *Pl. vancouverensis*. The contents of the vaso-peritoneal cells

TEXT-FIG. 16.

Vaso-peritoneal cells and ova on a capillary. ( $\times 200$ .)

appear to be identical with those already described for this species, similar observations having been made with regard to the white pigment-granules which in *Phoronopsis harmeri* are very noticeable (Fig. 16, *p. g.*).

There are numerous ova in various stages of development to be found in the vaso-peritoneal tissue on both sides of and below the alimentary canal in all the specimens of which the proximal ends have been cut, and they are surrounded by distinct follicle cells (Fig. 16, *ov.*, *fol.*). No spermatozoa have been seen, nor have any ova been observed either free in the body cavity or in the excretory ducts.

**Affinities.**—There can be no doubt that this animal is a species distinct from *Phoronopsis albomaculata*, the other member of the genus. The latter, from South Africa, described by Gilchrist (7), was 18 mm. long, and the tube was attached by one side to its substratum with the two ends near together. The lophophore was horseshoe-shaped and carried 126 tentacles. There were 94 fascicles of longitudinal muscles.

The comparatively enormous size, greater number of tentacles and different habit of life of *Phoronopsis harmeri* are, I venture to suggest, minor points of difference, and the shape of the lophophore and the possession of a far larger number of muscle fascicles are the more important systematic characteristics.

In conclusion I should like to thank the Rev. G. W. Taylor, F.R.S.C., Curator of the Marine Laboratory, Departure Bay, for his courtesy and help during my visit. I also wish to express my thanks to Dr. Harmer, F.R.S., for kindly giving me assistance, especially with regard to literature, and to Dr. Marett Tims for his help and interest throughout.

ZOOLOGICAL LABORATORY,  
BEDFORD COLLEGE,  
UNIVERSITY OF LONDON;  
March 5th, 1912.

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## **Transverse Segmentation and Internal Differentiation of Chromosomes.**

By

**W. E. Agar,**

Glasgow University.

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With Plates 12 and 13.

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THE material for this paper was partly the same as was used for my former paper on the spermatogenesis of *Lepidosiren*, partly *Lepidosiren* larvæ obtained during the same expedition, and partly larvæ collected by Prof. Graham Kerr on his previous expedition to the Paraguayan Chaco.

### **SOMATIC MITOSES.**

In my paper on the spermatogenesis of *Lepidosiren*, it was shown that the univalents of the diakinesis of the first meiotic prophase develop a very marked transverse constriction. When these univalents pair (i. e. the second pairing as described in the paper), the transversely constricted constituents of each pair form together a perfectly typical "tetrad." As has now been found to be the case in so many forms, the four segments of each tetrad are not distributed to the four gametes, but both divisions are longitudinal—that is, the transversely constricted chromosomes ("dyads") of anaphase I split longitudinally to form tetrads again, and anaphase II separates chromosomes still transversely constricted as they were in prophase I. These transverse constrictions are left, therefore, without any assignable significance. Attention was drawn in the paper alluded to to the probability that the transverse constrictions correspond with the apices of the V's

of the somatic or spermatogonial mitoses. Further evidence will now be produced in favour of this conclusion, and to show that this transverse segmentation is potentially present in all the chromosomes in all parts of the body, though it is most marked whenever the chromosomes are particularly short. In any larva of *Lepidosiren* occasional mitoses are found with unusually short chromosomes, and these were found to be extraordinarily numerous in one larva of Graham Kerr's stage 31 +. They are present in probably all the tissues, but especially in the nervous system.

Fig. 1 shows a metaphase in a nerve-cell from this larva. It is cut in three sections. It will be seen that the chromosomes are very short, and that the majority of them are markedly transversely segmented. As they are all completely longitudinally split also, the result is to form tetrads very similar to those found in meiosis, but present of course in the full somatic number (thirty-eight). The scattering of the chromosomes through the cell at this late stage is to be noted, and I believe that it is a frequent, though by no means invariable, characteristic of this type of mitosis that no equatorial plate is formed.

Figs. 2 and 3 are small fragments of two metaphases, also in nerve-cells, showing a few tetrads produced in the same way as those in Fig. 1.

In all these figures it is noticeable that certain of the chromosomes are divided by their transverse joints into very unequal portions.

Such figures as these could be multiplied indefinitely from the same larva, and from others.

The question now arises, at what stage does the transverse segmentation appear? As a rule, it seems not to do so till the metaphase, though there are numerous exceptions to this, as can be seen in certain chromosomes in fig. 8. Fig. 4 is a prophase of a somatic mitosis from the same larva as that from which figs. 1, 2 and 3 are taken. This prophase would undoubtedly have resulted in very short, and hence transversely constricted metaphase chromosomes, but nevertheless, no certain segmentation is yet visible in them.

Fig. 5 shows an anaphase, such as would presumably result from a metaphase like fig. 1. The irregular scattering of the chromosomes corresponds with the probable absence of a definite equatorial plate mentioned above. There is no reason to suppose that this irregularity of grouping leads to an unequal partition of the chromosomes to the daughter-nuclei.

Figs. 6 and 7 represent metaphase chromosomes from somatic mitoses with long chromosomes. Fig. 6 is part of a nucleus immediately before the chromosomes are placed on the spindle. Many of them are completely split into daughter-chromosomes, and some of them show little or no transverse segmentation. In certain of them, however (*a, b, c, d*), this is very apparent, and it is to be noticed that the point of segmentation corresponds with the apex of the V. It is striking, too, that in some, especially *d*, the division occurs much nearer one end than the other. It is true that in *d* the two short segments are a little affected by foreshortening, but this is not enough to account for more than a very small part of the difference in length between the two limbs.

Fig. 7 is a later stage in so far that the equatorial plate is fully formed, but nevertheless the chromosomes are not so completely split as in fig. 6. The indication of transverse segmentation is so slight that the fact that it is represented by the apices of the V's could not have been recognised without the help of the other figures.

A number of chromosomes from different mitoses in various somatic tissues of two larvæ are collected into fig. 8. A comparison of these with the foregoing figures will suffice to show the general nature of the segmentation in the somatic chromosomes.

The chief points to notice are:

(1) The frequent very complete separation of the two portions by the transverse joint. This separation often seems at first sight to involve the whole chromosome, but closer inspection shows that it involves only the chromatin. However separate the two chromatic portions may be, they are always joined by a bridge of non-staining substance. This

bridge may be as wide as the rest of the chromosome, or a mere thread.

(2) The frequent inequality of the segments into which the chromosome is divided.

(3) The fact that the metaphase split does not extend through the point of segmentation till after it is complete in the other parts of the chromosome. This leads to the assumption by the chromosomes of various shapes. In the case of long chromosomes it often leads to an appearance of two  $\vee$ 's connected by their apices by a thin strand (fig. 8, *c, e*, and fig. 6, *a, b, c*). Later, of course, the longitudinal split will extend through the connecting bridge also, and then an effect may be produced as in *n*, fig. 8. More often, however, in the case of long chromosomes the extension of the metaphase split through the connecting bridge is accompanied by a partial flattening out of the transverse constriction.

Another effect of the tardy splitting of the connecting bridge is the frequent occurrence of **X**-shaped chromosomes, some of which are shown in fig. 8 (*g, h, j, k*). Their mode of origin is obvious if compared with those chromosomes in which the daughter halves of the two end-to-end segments have not diverged so widely (fig. 8, *e*, and fig. 6, *a, c*).

#### SPERMATOGONIAL MITOSES.

In the newly formed spermatogonial equatorial plates the chromosomes attain a great length, and correlated with this there is little, if any, indication of transverse segmentation. Such an equatorial plate is shown in fig. 9. The same absence of transverse segmentation, or even of sharp bends at the apices of the  $\vee$ 's, is shown in fig. 6 of my former paper. As the chromosomes shorten, however, transverse segmentation becomes evident in many of them, and at the time that the metaphase splitting takes place it is often very pronounced, especially in the smaller plates. These are very difficult to analyse, as the confusion caused by the crowding together of the chromosomes far more than counter-balances the advantage

gained by their smaller size. The best examples are to be obtained from the still further shortened chromosomes of the daughter-plates. This is shown in fig. 7 of my former paper.<sup>1</sup>

The whole series of chromosomes from this nucleus is shown here in fig. 15 A.

Figs. 10-12 are spermatogonial daughter-plates, and a transverse joint is very clear in many of the chromosomes, especially in the smaller ones.

Attention has already been drawn to the fact that the transverse joint often divides the chromosome into two very unequal segments, and also that the apices of the V's of the longer chromosomes correspond with the transverse joints of the shorter ones. Special regard should be paid to figs. 10 and 11 and 15 A in this respect. In all of these it is seen that the two longest chromosomes form V's with approximately equal limbs, while those next in size have very unequal limbs. As we follow down the series of chromosomes the V's gradually pass into dumb-bells in the shortest ones.

#### MEIOTIC PHASE.

The development and fate of the transverse constrictions of the meiotic chromosomes was fully described in my former paper. Here it is only necessary to recall that there emerges from the synizetic mass the full number of long chromosomes, which become spaced out through the nucleus during diakinesis. At first (i.e. while they are still long) they are unsegmented, but as they shorten up the transverse constrictions appear. Finally they become more or less dumb-bell or hour-glass shaped, and then pair to form the typical meiotic tetrads.

It is unnecessary to describe the development of the transverse constrictions again here, so I have started with the fully formed tetrads. A complete series of these, from a cell

<sup>1</sup> In the explanation of the figures, and on p. 26 of that paper, this figure is referred to as an equatorial plate. This is a slip, and it is correctly described as a daughter-plate on p. 22.

in which the spindle is fully developed, but the chromosomes are not yet arranged on the equatorial plate, is given in fig. 15 *b*. A metaphase, or early anaphase, of the first meiotic division is shown in fig. 13. In *d* we see a tetrad ring, breaking simultaneously through both points of attachment of the conjugants. In *c* we see the more usual condition, where one point of attachment has given way before the other, and the ring has straightened itself out. In *a* and *e* the dissociation has gone further, the constituents of the tetrad remaining attached by a thin thread only.

The complete series of chromosomes from a nucleus at the same stage is given in fig. 15 *c*.

Fig. 14 shows two daughter-plates resulting from a first meiotic division. It is somewhat unusual for daughter-plates to be formed in this way in the meiosis of *Lepidosiren*, the chromosomes as a rule remaining bunched together near the two poles, while the spindles rotate for the second division. The longitudinal splitting preparatory for the second division is, therefore, exceptionally well shown in this figure. Each chromosome again forms a tetrad, owing to the transversely constricted chromosomes of anaphase I having each divided lengthwise.

The chromosomes forming the right-hand plate (some of which are in the next section) are shown in fig. 15 *d*. Only eighteen tetrads were present, one having evidently been carried away by the razor.

A consideration of the spermatogonial and meiotic figures at once shows a very important fact, namely that the transverse segmentation of a given chromosome always takes place at the same spot. In the case of the longer chromosomes the segmentation is marked by the bend of the V, in the shorter ones by a transverse constriction. This constancy as to the point of bending or constriction can also be gathered from the somatic mitoses, but partly owing to the greater complexity of the figures it is not possible to demonstrate it so clearly as can be done in the gonadic divisions.

In the four series of chromosomes shown in fig. 15 I have



tried to arrange the chromosomes roughly in order. The large pair, or large tetrad, can nearly always be easily recognised, but the gradation in size amongst the remainder is so continuous, and disturbances due to foreshortening, etc., so inevitable, that it is impossible to attain to accuracy of arrangement. Fortunately for our purpose, however, while the large pair of chromosomes always segments approximately symmetrically, at least the next four pairs in size segment very unequally. Hence, confining our attention to the few largest pairs only, we can see that among them at least the angles of the V's or transverse constrictions of the tetrads always occur in the same region of any given chromosome. While it is not possible to be sure of the identification of all the other chromosomes, an examination of the figure leaves little doubt in one's mind that the same constancy holds for the smaller chromosomes also. This impression is gathered still more strongly from the preparations themselves, in which it is easier to allow for irregularities due to foreshortening, etc.

The equality of the segments of the pair of large chromosomes and inequality of those next in size is shown incidentally in figs. 24-31 of my former paper.

Another important fact brought out by a study of the meiotic figures is that in the asymmetrical tetrads like ends are always applied to like. [That is, the small segment is applied to small and large to large]. In the case of the somatic tetrads this orientation follows from their mode of formation. The case of the meiotic tetrads is different. As described in my former paper, at the time that the transverse constrictions are developed, the chromosomes may be lying widely scattered through the nucleus. Hence the juxtaposition of like ends to like when they pair to form the definitive tetrads must be put down to an active cause.

#### LITERATURE.

Many cytologists have noted the occasional occurrence of "tetrads" or transversely segmented chromosomes in somatic

cells. Della Valle, in a paper in which he himself describes half-a-dozen nuclei with tetrads from various tissues of the salamander, and from Bidder's organ in the toad, has collected together a large number of references; Grégoire has added to the list, and from these two authors it may be seen how widely distributed such occurrences are throughout both the animal and vegetable kingdoms. It is interesting to note that Grégoire and Wygaerts figure three transversely segmented metaphase chromosomes of *Trillium*, in all of which they are divided by the joint into two unequal lengths, and that on p. 15 they state that the commonest form of anaphase chromosome in this plant "est celle d'un V incomplet, formé d'une grande et d'une petite branche."

Besides della Valle, very few authors have published researches directed specially to the occurrence of transverse divisions in somatic chromosomes. Popoff has described the appearance of tetrads in the liver-cells of *Paludina*, generally in the full somatic number. These are closely similar to the tetrads appearing in the primary oöcytes of the same animal, and Popoff (like della Valle) considers that in both cases they are due to a physiological abnormality of the cells in question.

An inquiry into somatic tetrads has been made by Häcker and his pupils. Häcker brought out the transverse segmentation of the chromosomes in developing Copepod eggs with diagrammatic distinctness by the action of ether. This has been done still more beautifully by Schiller, working on several species of *Cyclops*. His figures 7-15 show typical tetrads, present of course (except in rare cases) in the somatic number.

Němec has brought about the formation of tetrads in somatic plant tissues by the action of chloral hydrate.

This brief reference to the literature will be enough to show that the tendency to transverse segmentation of chromosomes is very widely distributed throughout the animal and vegetable kingdoms—probably, indeed, the potentiality to such segmentation is present in all chromosomes, and becomes

operative especially often when they become, for any reason, unusually short in proportion to their length. In this case the transverse constriction together with the metaphase split gives the well-known tetrad appearance.

The only constructive theory as to the significance of this tendency to transverse segmentation that is known to me is Häcker's theory of teleutosyndesis. It is unnecessary to dwell long on this, however, as it seems very improbable that it can be maintained in the face of the facts brought forward in this paper. For, firstly, the theory requires the final fusion of the end-to-end segments at some stage in the life-cycle to prevent the number of segments in each chromosome being doubled in each meiosis. It is extremely improbable that such a fusion ever takes place in *Lepidosiren*, as the "doubleness" is traceable in all the tissues of the animal, and is specially well marked in the daughter-plates of the spermatogonial divisions—that is, in the last divisions before the meiotic prophase where the end-to-end conjugation is supposed to take place again. Secondly, the constant inequality of the two segments of many chromosomes is very much against the theory that they have been formed by the permanent pairing end-to-end of pairs of homologous ones. Thirdly, the theory is in any case of such a nature as could only be accepted on very strong direct evidence in its favour.

#### GENERAL CONSIDERATIONS AS TO THE NATURE AND SIGNIFICANCE OF THE TRANSVERSE SEGMENTATION.

The immediate cause of the transverse segmentation may well be physical—perhaps the tendency may be for the chromatin to collect at the ends of the chromosomes and flow away from the middle. This is possibly dependent on purely physical factors—perhaps electrical charges or surface tension, both of which have often been supposed to play a large part in the movements observable in mitosis. In accordance with this view is the fact that the constrictions are best

marked when the chromosomes are shortest. This has been alluded to several times already, and for definite data I refer to fig. 15 A, and to the description of the development of the transverse constrictions in the shortening chromosomes of the meiotic prophase in my former paper.

If the chromosomes always segmented in the middle, or, failing that, at no definite spot, the matter would possess but little interest. But the fact that the segmentation always occurs in the same chromosome at the same spot demonstrates that the chromosomes possess a constant lengthwise differentiation. For if a chromosome were homogeneous, or if its internal differentiation were not constant, a transverse constriction developed by physical or other means would either always occur in the middle or else at no constant spot.

The theoretical importance of inductive evidence of this differentiation needs no emphasis. We learn, of course, very little about the nature of the differentiation. It may consist only in a physical or chemical difference between the chromatin at the two ends of the chromosome; or it may be that, as demanded by theory, the differentiation lies in the chromomeres of which the prophase chromosome is composed, and that when the tug comes the chromosome always gives way between that pair of chromomeres (always situated in the same spot in the chromosome) which are least firmly attached to one another. An indefinite number of other possibilities present themselves, but all demand the hypothesis that a given chromosome is always composed of the same differentiated portions arranged end-to-end in the same order along its length.

The fact that in the diakinetik pairing of asymmetrically divided chromosomes to form the definite tetrads, like ends are always applied to like, is another indication of a constant differentiation, which is only made apparent by the probably physical factor, which produces the visible segmentation. Moreover it implies that there exists an attraction between like parts of homologous chromosomes, not only between such

chromosomes as a whole.<sup>1</sup> The permanent forms of meiotic chromosomes found in certain animals by Moore and Arnold, and by Walker, may reasonably be supposed to be due to the same lengthwise differentiation.

Finally, the facts here described are plainly in favour of the theory of chromosome individuality. Quite recently Meves has attacked this theory, largely on the grounds that though size differences exist amongst the chromosomes of the salamander, yet individual variation, and unavoidable disturbances due to bending, foreshortening, etc., are so great as to make impossible the constant identification of the same chromosome. He is also sceptical of the possibility of arranging the somatic chromosomes in pairs. Such negative evidence as his cannot, however, be held to counterbalance positive evidence gained from those forms in which the size and other differences are great enough to appear through all disturbing factors. This is certainly the case with, at any rate, the large pair of chromosomes in *Lepidosiren*.

#### SUMMARY.

(1) The tendency for chromosomes to become transversely segmented or constricted is a wide-spread characteristic. It becomes operative especially, but not solely, whenever the chromosomes are short in comparison with their length, as happens normally in meiosis, and exceptionally in somatic tissues.

(2) The point at which the constriction or segmentation takes place in any given chromosome is constant for that chromosome, and is the same as the point at which it most readily bends to form the angle of the  $\nabla$  when present in that form.

<sup>1</sup> Accepting the commonly held views as to chromosome individuality and reduction. Those who would hold the opinion that the scattered chromosomes of the meiotic prophase are not whole chromosomes, but precociously separated daughter halves, have still to explain the fact that when they come together again on the equatorial plate, like ends are always applied to like.

(3) The constancy of the position at which transverse segmentation takes place indicates a constant differentiation of the chromosomes in a lengthwise direction.

(4) The presence of transverse constrictions or joints in chromosomes is not, without special evidence, to be taken as an indication of bivalency, or of a future division plane.

#### EXPLANATION OF CERTAIN TERMS.

At the request of the Editor, I take this opportunity of reminding readers that Wilson's 'The Cell in Development and Inheritance' contains an excellent glossary. The few distinctively cytological terms used above which are not to be found there or in the index of that book are given below.

Diakinesis (V. Häcker, 1897).—The stage in the meiotic prophase following synizesis, in which the chromosomes are scattered widely apart in the nucleus.

Meiosis (J. B. Farmer and J. E. S. Moore, 1905).—The phase in which reduction of chromosomes takes place, including both maturation divisions. It is convenient to designate the stages of the first and second divisions as metaphase I, metaphase II, etc. (Grégoire).

Somatic should be restricted to mitoses in the cells of the body outside the germ-track. By some authors this term is applied to all mitoses outside the meiotic phase, including, therefore, those of the spermat- and oo-gonia, but this misuse of the term is to be deprecated.

Synizesis (C. E. McClung, 1905).—The clumping together of the chromatin often observed in the meiotic prophase. It was included in Moore's term, "synapsis."

Teleutosyndesis (V. Häcker, 1910).—A theory of chromosome conjugation, according to which the conjugants are permanently united in the meiotic prophase.

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## EXPLANATION OF PLATES 12 AND 13,

Illustrating Dr. W. E. Agar's memoir on “Transverse Segmentation and Internal Differentiation of Chromosomes.”

[All figures were drawn with the Abbé camera, under a magnification of 2500 (Zeiss 1.5 mm. Apochr., 12 comp. oc., drawing table at the level of the microscope stage). In reproduction, all figures have been reduced to  $\frac{2}{3}$  except fig. 15, which is reduced to  $\frac{1}{3}$ . Final magnification of figs. 1-14, as reproduced, is therefore about 1875. All the figures are of *Lepidosiren paradoxa*.]

### PLATE 12.

Fig. 1.—Metaphase, cut in three sections from a nerve-cell. Larva of Graham Kerr's stage 31 +. Sublimate-acetic.

Fig. 2.—Portion of a metaphase in a nerve-cell of the same larva.

Fig. 3.—Portion of a metaphase in a nerve-cell of the same larva.



Fig. 4.—Portion of a late prophase from a mesenchyme cell in the same larva.

Fig. 5.—Anaphase, nerve-cell of same larva.

Fig. 6.—Chromosomes about to be placed on the spindle. Muscle-cell, larva of stage 32 +. Sublimate-acetic.

Fig. 7.—Polar view of equatorial plate from same larva. Only a few of the chromosomes are shown.

Fig. 8.—Chromosomes from various tissues of two larvæ (stages 31 and 31 +). Sublimate-acetic.

Fig. 9.—Polar view of an unsplit equatorial plate of a spermatogonium. This is intact, in a  $35\mu$  celloidin section. Sublimate-acetic.

Fig. 10.—Portion of anaphase of spermatogonial division. The two large chromosomes are partially overlying one another. Sublimate-acetic.

Fig. 11.—Portion of late anaphase of spermatogonial division. Sublimate-acetic.

### PLATE 13.

Fig. 12.—A pair of daughter-plates of a small spermatogonial division. 12 *b* has some chromosomes missing, 12 *a* is intact. The two plates were in the same  $40\mu$  celloidin section, mounted between two cover-slips, and the figure was obtained by drawing the upper one from one side, then turning the slide over and drawing the other one from the other side. The two large chromosomes are shown in 12 *a*, but one limb of one of them is very much foreshortened. Sublimate-acetic.

Fig. 13.—Portion of metaphase, first meiotic division. The large tetrad is not shown. Flemming.

Fig. 14.—Pair of daughter-plates from a first meiotic division. Not all the chromosomes are shown. Sublimate-acetic.  $40\mu$  celloidin.

Fig. 15.—Entire series of chromosomes from four nuclei of different stages: A. Spermatogonial daughter-plate. B. Tetrad rings just prior to formation of the equatorial plate of the first meiotic division. C. Metaphase, first meiotic division. D. Tetrads from daughter-plate of first meiotic division (the right-hand plate of fig. 14.) The nucleus A is intact in a  $40\mu$  celloidin section. B, C and D are all cut in two sections, but probably in no case is any chromosome cut. In D one of the tetrads has evidently been carried away by the razor. In B and C the tetrads are orientated as they would be if the spindle axis were parallel with the top edge of the page. \* Shape of chromosome seriously affected by foreshortening. † Chromosome partially concealed by other chromosomes, so that accuracy is not certain. (See fuller description in text, p. 291.)







FIG 1



FIG 2.

FIG 3.



FIG 4

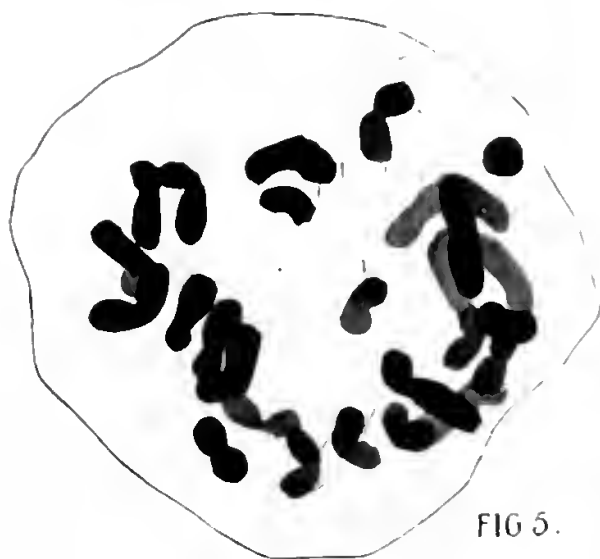


FIG 5.



FIG 6.



FIG 7.

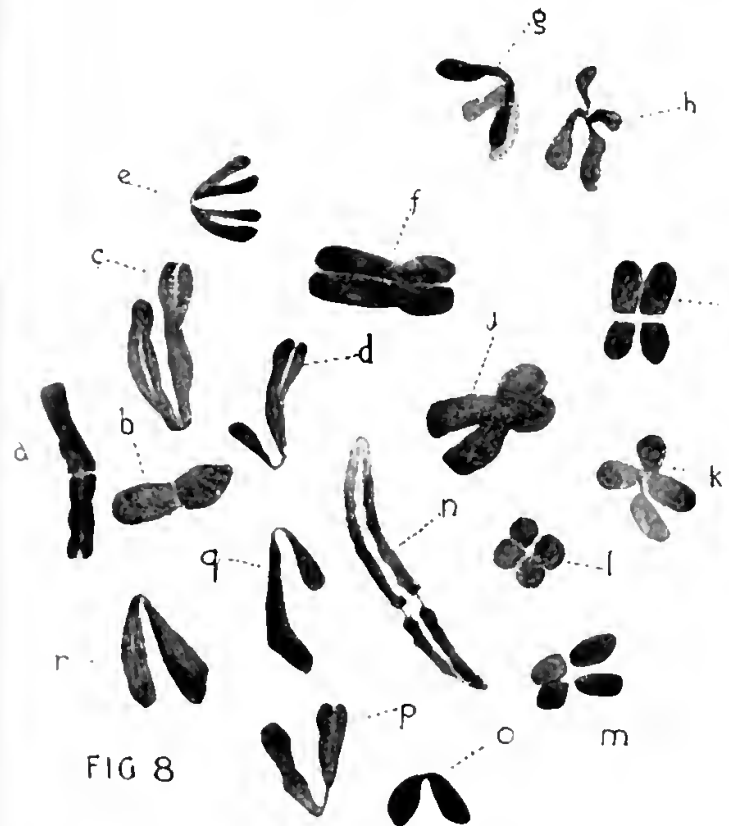


FIG 8

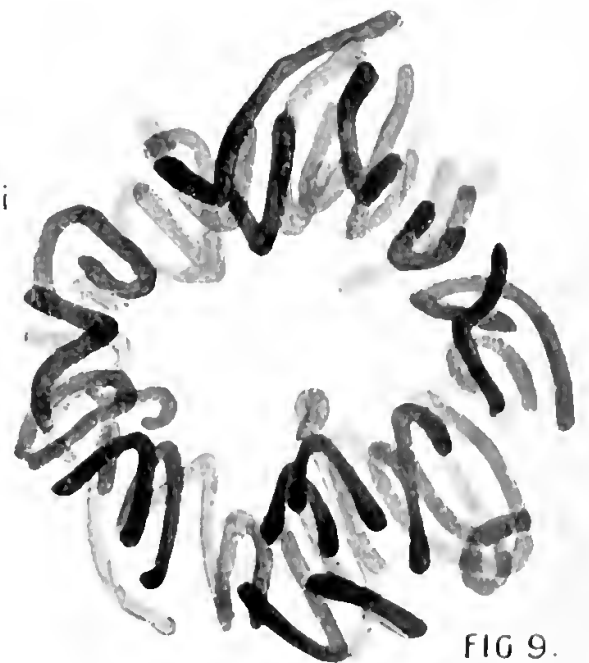


FIG 9.



FIG 10.



FIG 11.





FIG 12.

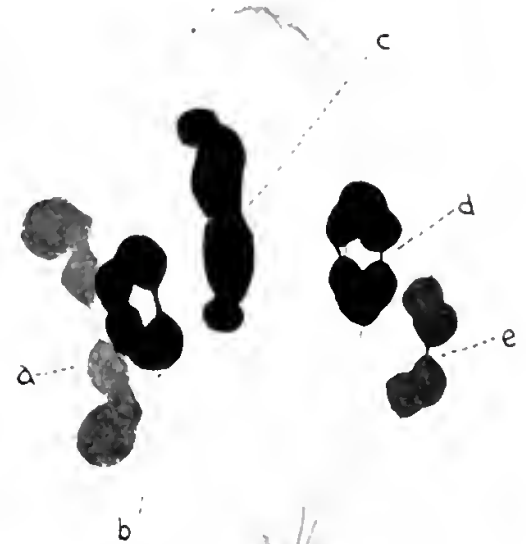
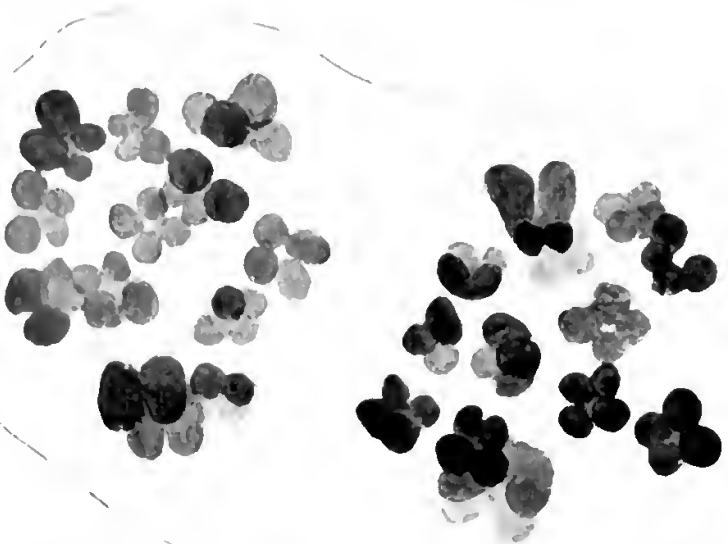


FIG 13.

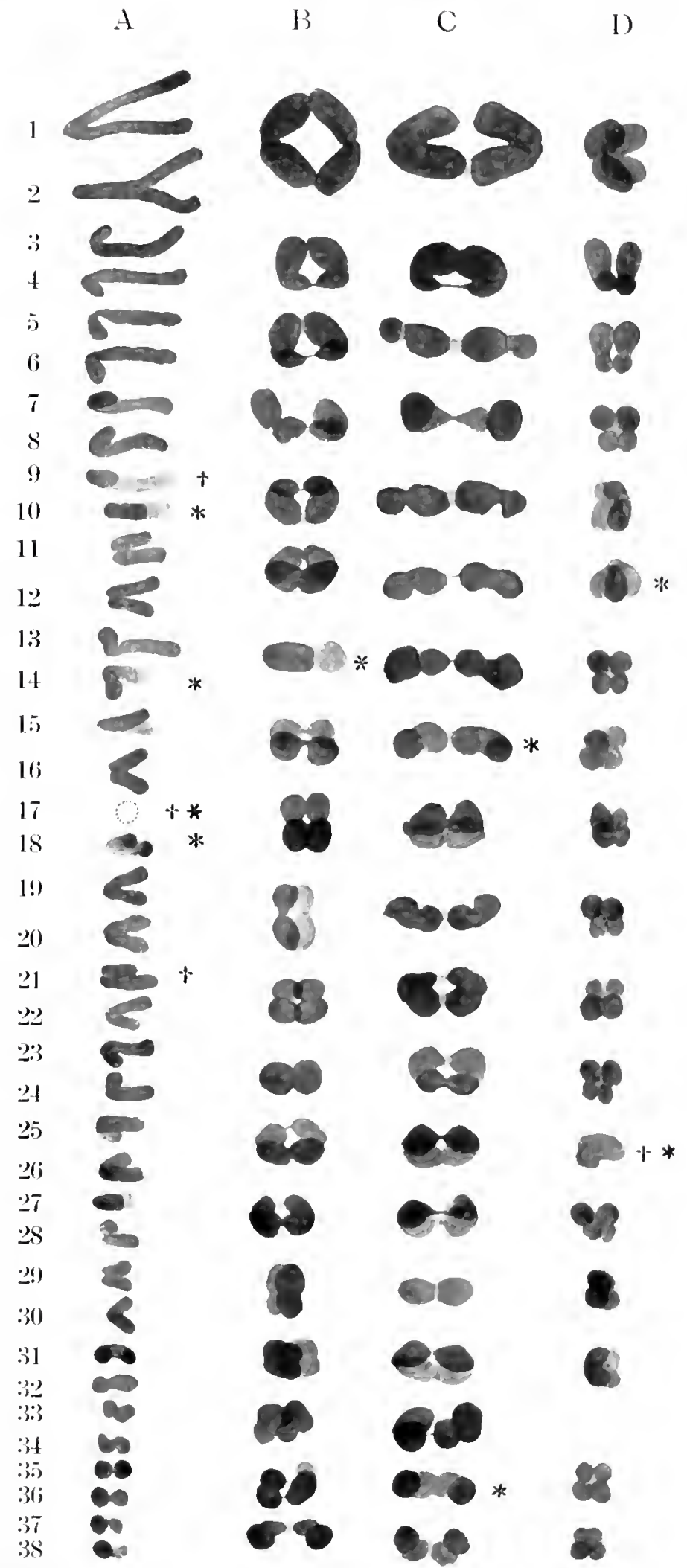
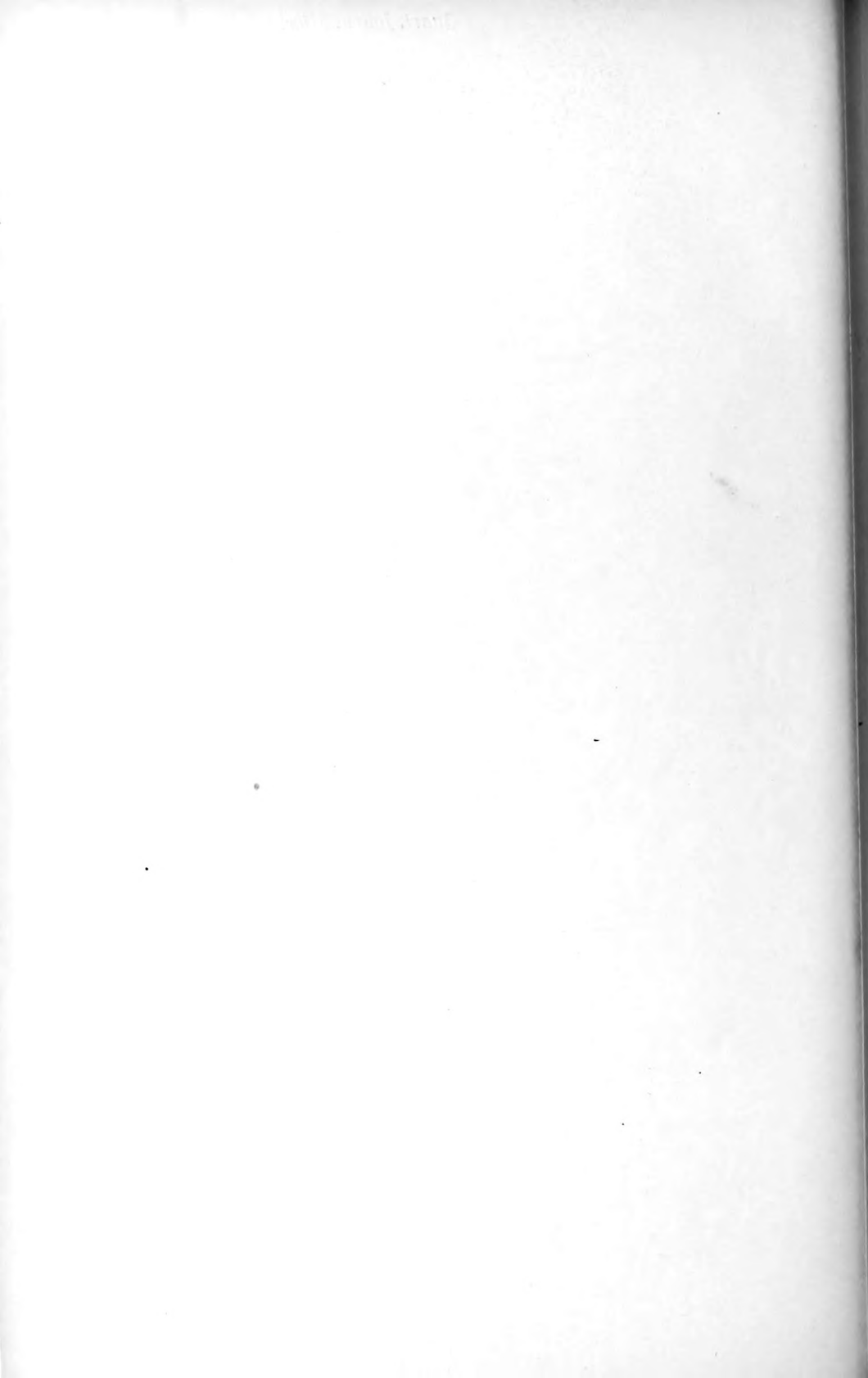


FIG. 15.









## Studies on the Development of Echinoidea.

### II. The Early Larva of *Echinocardium cordatum* and the Result of Crossing this Species with *Echinus esculentus*.

By

**Prof. E. W. Macbride, D.Sc., LL.D., F.R.S.,**  
Imperial College of Science and Technology, South Kensington.

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With Plates 14 and 15.

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THE results recorded in this paper were obtained during two months' sojourn at the Biological Station of the West of Scotland Marine Biological Association at Millport last summer. A preliminary account of the same has already been published in the 'Proceedings of the Royal Society' (16).

I have to record my thanks to Mr. Richard Elmhirst, Director of the Station, for the whole-hearted manner in which he aided my endeavours, and to Dr. James F. Gemmill, Vice-President of the West of Scotland Marine Biological Association, for the assistance he rendered me in providing me with pure cultures of diatoms, which were invaluable as food for the developing larvæ. To Prof. Graham Kerr, F.R.S., and to Dr. Agar, of Glasgow University, my best thanks are due for the loan of apparatus from the Zoological department of that University. And finally, my warmest thanks are due to my friend, L. W. Byrne, Esq., who re-drew my figures for me so as to make them suitable for publication.

The main object of my research was to test the distribution of paternal and maternal characters in the hybrid produced by crossing two species, in whose larvæ distinctly specific characters could be found.

A great deal of work has been done in crossing distinct

species of Echinoidea, and most contradictory results have been arrived at, as a short review of the principal papers on this subject will show.

Interest in the question of the character of the hybrid larvæ produced by crossing two species of Echinoidea was first awakened by two remarkable papers of Boveri (1, 2). In these papers, which record the same researches, he describes the effect of fertilising fragments of *Sphærechinus* eggs with spermatozoa derived from *Echinus*. He found that he obtained some hybrids of purely maternal type, others of mixed character, and some very small ones of purely paternal type. These last he attributed to the development of non-nucleated fragments of eggs which had been entered by spermatozoa of *Echinus*, since he had previously proved that non-nucleated fragments of eggs could develop into larvæ when entered by the spermatozoa of their own species. He therefore concluded that the nucleus alone was the bearer of heredity, since it could impress an *Echinus*-character on a fragment of a *Sphærechinus* egg.

This conclusion was attacked by Seeliger (17, 18), who confirmed Boveri's statement that enucleated fragments of eggs could be fertilised by sperm of the same species, but who denied that any hybrids figured by Boveri had been derived from such enucleated fragments, since, in a normal hybrid culture, larvæ of every type occur—from those showing a purely maternal character to those which closely approximate to the paternal type. Therefore he concluded that Boveri's inference that the nucleus alone was the bearer of the hereditary qualities was not sustained by his experiments. Seeliger also pointed out that Boveri had not properly described the typical form of the larva in each of the parent species, and that no valid conclusion as to the character of the hybrid could be drawn until this had been done.

Driesch (5) then took up the subject, and pointed out that in crosses of *Sphærechinus* and *Strongylocentrotus* *Echinus* the hybrids could be of purely maternal type in respect of (A) rapidity of development, (B) number of mesen-

chyme cells, (c) general form. But the first thorough investigation of the characters of the larvæ of the genera *Echinus*, *Strongylocentrotus* and *Sphærechinus* and of the hybrids produced by crossing these species was made by Vernon (23, 24). It must be borne in mind that it is possible to fertilise the eggs of *Sphærechinus* with the sperm of *Echinus* and *Strongylocentrotus*, but that eggs of *Echinus* or *Strongylocentrotus*<sup>1</sup> generally refuse to develop when fertilised by the sperm of *Sphærechinus*. Vernon came to the general conclusion that the predominance of paternal or maternal characteristics in the hybrid offspring was a question of the relative sexual ripeness of the male and female parents.

It may be incidentally remarked that the larvæ of *Echinus* and of *Strongylocentrotus* are not separable from one another by any clearly defined characters, but that those of *Sphærechinus* are sharply separated from the others by having the skeleton supporting each of the two post-oral arms in the form of a lattice-work consisting of several parallel calcareous rods joined to one another by numerous cross-bars. The skeleton of the post-oral arms of the other genera consists simply of single bars. It follows that the main feature relied on in determining which parent's influence predominates in the hybrid is the character of the skeleton of these arms.

Vernon's work was criticised in 1902 in a remarkably good paper by Steinbrück (20), in which he dealt with the cross between *Strongylocentrotus* and *Sphærechinus*. Steinbrück called attention to the fact that in their so-called distinctive characters the larvæ of both forms are variable; that in pure cultures of *Strongylocentrotus* larvæ are occasionally met with which possess two calcareous rods with occasional junctions between them in the post-oral arms, and that in pure cultures of *Sphærechinus*, larvæ are sometimes found in which the lattice-work is partly abolished in

<sup>1</sup> Driesch (5) records that 1 per cent. of *Strongylocentrotus* eggs develop when fertilised with *Sphærechinus* sperm.

the skeleton of the same arms; and further, that the hybrids between these two species are of a very variable character, so that from them a complete chain of forms can be selected leading from larvæ showing purely paternal to those showing purely maternal characters.

Steinbrück's work has not received the attention which was due to it. It was almost ignored by the next workers who occupied themselves with the subject—Doncaster and Herbst. Doncaster (4) admits that variations occur in the characters exhibited by pure cultures of *Strongylocentrotus* and *Sphaerechinus*, but believes them to be relatively so infrequent that they may be disregarded, and he bases his estimate of the relative intensity of paternal and maternal influence exhibited in the character of the hybrid larvæ on the same criteria as those employed by Vernon. He arrives at the conclusion, however, that the influence of either parent—the father, for instance—does not vary with the ripeness, immaturity or staleness of the sexual products, but with the temperature of the water, for he found that he obtained the same results in December as in May if he artificially warmed the water used for the December cultures. This he explains by stating that warmed water causes the stronger larvæ to develop rapidly until they reach the point of development where further progress depends on food. Then they die and the weaker and more slowly developing larvæ survive them, and it is these that show the paternal influence most. In colder water the stronger larvæ develop more slowly, and hence are present at the time when the estimate is made (eight days after fertilisation).

Boveri (3) returned to the subject of the characters exhibited by the bastards produced by crossing *Strongylocentrotus*, *Echinus* and *Sphaerechinus*. He maintains that the influence of the male parent is visible in the shape, skeleton, pigmentation, mesenchyme cells, and sometimes the size of the hybrid larva. To this Driesch replied (6), controverting all these points except the one concerning pigmentation.

Herbst, in a most elaborate paper (10), in which he deals

with the result of fertilising the eggs of *Sphærechinus* with the sperm of *Strongylocentrotus* and of *Echinus*, arrives at much the same results as Doncaster, but he admits that there is also a factor independent of temperature which determines the greater or less predominance of paternal characters, and this he finds in the varying character of the eggs. In a subsequent paper (11) he records the results of the experiment of initiating parthenogenesis in the eggs of *Sphærechinus* by treating them for a short period with valerianic acid and then fertilising them with the sperm of *Strongylocentrotus*. Under these circumstances he claims that he obtained a displacement of the development in the maternal direction, and he maintains that in a few cases he even obtained a larva maternal on the one side of the body and paternal on the other—a circumstance which he accounts for by supposing that the spermatozoon had entered the egg after the egg-nucleus had divided and had united with one of the two daughter-nuclei so formed.

Fischel (7) a little later dealt with the hybrids produced by crossing *Arbacia* and *Sphærechinus* and also *Arbacia* and *Strongylocentrotus*. (It is to be noted that he persistently and erroneously uses the term *Echinus brevispinosus* for *Sphærechinus granularis*). He arrived at results of generally the same character as those gained by Driesch; but he tries to show that the effect of foreign sperm entering the egg is to interfere with the normal distribution of pigment in the egg. He asserts that the spermatozoon can influence the rapidity of development, the form and size of the larva, the development of pigment, the skeleton and the histology of the cells.

Tennent (22) tried the experiment of crossing the American forms *Toxopneustes* and *Hipponoe*. The larvæ of *Toxopneustes* are like those of *Strongylocentrotus*. Those of *Hipponoe*, on the contrary, resemble those of *Sphærechinus*. The cross could be made by using *Hipponoe* as male or as female parent, but in either case the larvæ showed the influence of *Hipponoe* as evidenced by the "lattice-work" in the skeleton



of the post-oral arms. Tennent's most remarkable conclusion was that the *Hipponoe* influence in the hybrid could be decreased and the *Toxopneustes*' influence correspondingly increased by decreasing the alkalinity of the sea-water by the addition of a few drops of very dilute acid.

Hagedoorn (9) crossed two species of the same genus, viz. *Strongylocentrotus purpuratus* and *Strongylocentrotus franciscanus*, and arrived at the conclusion that the hybrid was in every case purely maternal in its character, but in a paper published a little while after (12) by Loeb, Redman King and Moore recording the results of hybridisation experiments between the same two species, these authors state that they are unable to confirm Hagedoorn's results, but arrive at the curious conclusion that certain characters appear in the hybrid whichever way the cross is made—are, in fact, a dominant over-correlative characters; so for instance the clavate aboral ends of body rods are dominant over the pointed form, the spherical form of larva over the pyramidal, the presence of the recurrent rod in the skeleton over its absence and so on.

The reader will gain from the preceding review an impression of results of a most unsatisfactory and contradictory character as the fruit of the work of all these experimenters. Having had considerable experience in rearing the normal larvæ of Echinoderms, it struck me that one principal cause of such discordant results was the great variability of the characters relied on as distinctive of the different species of larvæ.

I therefore looked about for "species" to experiment on whose larvæ were distinguished from one another by clear and unmistakable characters. Two possible cases presented themselves to mind: first the case of the species *Echinus esculentus* and *Echinus miliaris*, and second, the case of *Echinus esculentus* and *Echinocardium cordatum*.

With regard to the first case, it should be noted that the differences between the larvæ of the two species do not become clear until the larvæ are about a month old and have

developed all eight arms. These differences concern the arrangement of the ciliated epaulettes, and the appearance of a green pigment in the larvæ of *Echinus miliaris*; they were described by me in 1899 (15). If anyone unacquainted with the details of the normal development of Echinoderm larvæ were to read through the papers of all the workers on hybridisation he would never suspect that the *Echinopluteus* larva ever developed more than four arms; and in this circumstance alone a strong instance is afforded of the necessity of knowing the ordinary development of a species before we make it the subject of "experimental embryology." In his first paper Boveri actually described the larva of *Sphærechinus* as possessing only two arms! Whilst I was seeking an opportunity to begin work the questions of the result of hybridising *Echinus esculentus* and *Echinus miliaris* was taken up by Shearer, De Morgan and Fuchs, who published a preliminary note of the results of their work (19). In this note they maintain that the hybrid between these two species is of a purely maternal character with respect to whatever character be selected for examination; and they maintain that this maternal character is not altered by changing the acidity or alkalinity of the sea-water employed. This certainly is a remarkable result to obtain by hybridising two species of the same genus, and experiments which have been made at the Imperial College, the results of which will shortly be published, do not bear out the view of these authors.

The second case forms the subject of the present paper. Vernon (25) had already recorded the results of crossing the eggs of two species of *Echinocardium* with the sperm of *Arbacia*, *Echinus*, *Sphærechinus* and *Strongylocentrotus*, and he found that the hybrid larvæ were all of a purely maternal type. When the sperm of *Echinocardium* was used to fertilise the eggs of the other genera no result was obtained except in one instance with the eggs of *Echinus*. In this case one third of the eggs developed and produced larvæ of a purely maternal type. The great distinguishing feature of the larvæ of *Echinocardium*, which it shares with the larvæ

of other Spatangoidea, is the possession of an aboral process of the body supported by a special skeleton. This, according to Vernon, is only developed on the fifth day, but according to him it appears in all the hybrid larvæ, although in them it is shorter than in the normal larva. Tennent (21), in a short preliminary notice read before the International Congress of Zoology held in Boston in 1907, had already announced that he had crossed the eggs of *Moiria*, a Spatangid, with the sperm of *Strongylocentrotus*. He gives no description of the hybrids, however, but in a later paper gives more details (23). He succeeded in fertilising the eggs of *Moiria* with the sperm of *Toxopneustes*. He kept the larvæ alive for seven days. None of them developed the aboral spine. Nevertheless, he says that they were of the "maternal intermediate type." He also made the reciprocal cross and obtained similar "intermediate" larvæ! Some were of the purely maternal type and developed for twenty days, but the possibility is not excluded that these were developed from eggs fertilised by chance spermatozoa of their own species.

Since the results which I obtained differ markedly from those recorded by Vernon, and are in many respects different from those obtained by Tennent, it may be worth while to give some account of the methods employed. At Millport large numbers of both *Echinus esculentus* and of *Echinocardium cordatum* were available. The former species could be picked off the rocks a stone's throw from the laboratory at ordinary low tides; the latter species could be obtained by the bucket-full at low spring tides by digging in a sandy beach about half a mile from the laboratory. The tank-water in the laboratory was unpolluted by drainage of any kind, for the laboratory is situated over a mile and a half from the centre of the little town of Millport, and the full tidal current of the Firth of Clyde sweeps past the rocks on which it is built. In the water in the tanks the adults of both species live comfortably, but it is, nevertheless, inimical to the larvæ. The reason of this is to be found in the metallic

pipes through which it is poured into the tanks; it is only necessary to dip up the water from the Firth in an earthenware or glass vessel in order to provide the larvæ with a medium in which they can grow and flourish. The eggs of both species when shaken out of the ovaries are provided with a glassy chorion which makes fertilisation difficult, but if the eggs are allowed to stand in clean sea-water for an hour two before being fertilised this membrane disappears. As tested by controls in which the eggs of each species were fertilised by its own sperm, it was evident that the material used was of the healthiest kind. The eggs of *Echinocardium* fertilised with their own sperm ran through their complete larval development and metamorphosed into young urchins in great numbers, accomplishing the whole cycle in three weeks. A full account of the normal development of this species is reserved for another paper. In the case of *Echinus esculentus* the larvæ lived for three weeks, developed ciliated epaulettes and all eight arms, but as I had previously given a very full account of the normal development of this species I gave no further heed to the larvæ. My success in rearing the larvæ I attribute to the cultures of diatoms provided by Dr. Gemmill.

When the eggs of *Echinocardium* were treated with the sperm of *Echinus* a moderate number of hybrids were produced, although possibly not more than one egg in a thousand developed. These hybrids lived for eight days, but then, in spite of being surrounded by abundance of food, they died. When the eggs of *Echinus* were treated with the sperm of *Echinocardium* about an equal number (one in a thousand) developed, and the resulting larvæ were of a purely maternal type. When, however, the sea-water in which the fertilisation was accomplished was previously sterilised by being heated to 70° C. no single *Echinus* egg developed, and when it was found that a certain number of *Echinus* eggs would develop if allowed to stand in clean sea-water without the addition of any sperm at all, it became

obvious that the supposed hybrids were really normally fertilised eggs whose fertilisation had been brought about by the accidental and unsuspected presence of spermatozoa of *Echinus* in the water employed. One cannot help wondering whether some of the results recorded in the literature, cf. that of Driesch that 1 per cent. of *Strongylocentrotus* eggs develop when fertilised with the sperm of *Sphæerechinus*, may not be due to a similar source of error.

Once this source of error had been detected all further experiments in cross-fertilisation were made in sterilised seawater, and every adult urchin before being opened was carefully washed in fresh water in order to destroy any spermatozoa which might be adherent to the outside of the test. The instruments employed were also carefully sterilised after each urchin had been opened before another was taken in hand. New experiments under these conditions gave exactly the same results when *Echinocardium* eggs were fertilised with the sperm of *Echinus*, and I consequently feel complete confidence in the accuracy of the results so obtained, but when the eggs of *Echinus* were fertilised with the sperm of *Echinocardium* no single egg developed.

Several authors, notably Loeb (12) and Godlewski (8), have succeeded in fertilising the eggs of sea urchins with the sperm of animals belonging to distinct classes, even to distinct phyla of the animal kingdom of Crinoidea, Mollusca, etc. In the case of Mollusca it has been proved that the sperm nucleus does not unite with the egg nucleus, i.e. the chromatin which the former brings into the egg is not included in the first karyokinetic spindle. In the case of Crinoids, however, Godlewski asserts that sperm and egg nucleus do unite, and that the chromatin of both is included in the formation of the first spindle. In all such cases of heterogeneous fertilisation the larvæ are of a purely maternal type and show no trace whatever of the paternal influence, and therefore this kind of development is termed "Entwicklungs-erregung," on the supposition that it is due to a

chemical influence exercised by the entering spermatozoon on the egg analogous to the action of salts in producing artificial parthenogenesis and having no relation to the ordinary hereditary action of the spermatozoon. The method employed to bring about this anomalous development is to add a small quantity of dilute alkali to the water in which the heterogeneous fertilisation is effected. Godlewski found that the

addition of 2.5 c.c. of  $\frac{n}{10}$  solution of NaOH was most effective

in producing results. I therefore tried the effect of fertilising the eggs of *Echinus* with the sperm of *Echinocardium* in vessels of sea-water, to which .5 c.c., 1.0 c.c., 1.5 c.c.,

2.0 c.c. and 2.5 c.c. respectively of  $\frac{n}{10}$  solution of NaOH had

been previously added to every 100 c.c. of sea-water employed.

In the mixture of 2 c.c. of  $\frac{n}{10}$  NaOH to 100 c.c. of sea-water a few unhealthy granular blastulæ were observed. In none of the other mixtures did a single egg develop.

Now Loeb has shown (13) that it is possible to get the eggs of sea urchins to develop if they are treated for a very brief period with a weak solution of butyric acid, then washed in sea-water, and then placed for an hour or so in sea-water rendered hypertonic by the addition of a few cubic centimetres of  $\frac{n}{10}$  solution of NaCl to every 100 c.c. of water.

The effect of the butyric acid is to cause the eggs to form membranes closely similar to those formed by eggs when normally fertilised. Development then begins, but if the eggs are not subsequently placed in hypertonic sea-water they break up into spheres which resolve themselves into smaller spheres and the whole egg is thus reduced to a heap of granules. Now if the actual formation of a vitelline membrane be carefully watched it will be found that the first step in this process is the formation at the surface of the egg of a large number of minute spherules whose outer walls coalesce to form the membrane. Hence Loeb puts

forward the view that the chemical action of the spermatozoon in provoking development consists of two parts; first a process of cytolysis is set up in virtue of which a vitelline membrane is formed, but this process would lead to the destruction of the egg if it were not checked; accordingly a second chemical action sets in in virtue of which the first action is arrested. In artificial parthenogenesis the checking of the process of cytolysis is effected by the hypertonic sea-water.

I was curious to see what actually had happened to the *Echinus* egg when treated with the sperm of *Echinocardium*. Microscopical examination revealed the fact that the eggs had formed fertilisation membranes, showing that the spermatozoa had entered them, but that they had then undergone cytolysis. One such egg is shown in fig. 1, Pl. 14. The spermatozoa of *Echinocardium* were therefore able to produce a cytolytic action, but incompetent to check it when it went too far.

Here, then, the cause of the sterility of the cross has been unmasked.

Turning now to the experiments in which *Echinocardium* eggs were fertilised with *Echinus* sperm I shall divide what I have to say into three parts. First I shall describe carefully the normal development of *Echinus esculentus* up till the sixth day; then I shall describe that of *Echinocardium cordatum* up till the fourth day; and then I shall describe the development of the hybrids, which, although they lived for eight days, did not attain a greater degree of development than that reached by the normal larvæ in about five days.

#### (1) THE DEVELOPMENT OF *ECHINUS ESCULENTUS*.

On the morning following the fertilisation of the eggs, which had been accomplished in the previous afternoon, clear spherical blastulæ (Pl. 14, fig. 2) were seen swimming at the surface of the water. The vegetative pole was already marked by the appearance of the first mesenchyme cells, but they had not yet migrated into the blastocele. Soon the blastula



became flattened on the vegetative pole and the mesenchyme cells wandered inwards. The majority of them formed a ring round the periphery of the flattened surface, but some wandered up the sides of the blastula and even reached the animal pole. At this pole there is a tuft of specially long cilia. The invagination which forms the archenteron began at the vegetative pole, and the ring of mesenchyme cells became thickened at two spots diametrically opposite to one another, and in these thickenings the rudiments of the larval skeleton appeared as two trifold "stars." Each arm of each star grew out, the growth being caused by the deposition of calcareous matter by the mesenchyme cells which cling to the arm. One arm of each "star" grows upwards and outwards—this is the rudiment of the post-oral rod of the skeleton; one arm grows backwards towards the opening of the invagination or "blastopore" and is the rudiment of the future "body rod" of the skeleton, and one grows horizontally across and forms the "horizontal rod" of the skeleton.

The invagination now deepens and forms the archenteron and the larva is now termed a gastrula. From the apex of the archenteron a single transversely elongated vesicle is cut off, which is the rudiment of the coelom. This stage, reached in two and a half days, is represented in Pl. 14, fig. 3. At the animal pole of the embryo a tuft of specially long cilia (*cil.*) is to be seen.

In the course of the next day the stomodæum makes its appearance as a shallow pit on one side of the anterior end of the larva. The side of the larva on which it appears becomes concave and constitutes the future ventral surface. The pit rapidly deepens and reaches the apex of the archenteron, with which it unites, and thus the alimentary canal is complete. The coelomic sac has just previously divided into right and left halves. From the "star" on each side another branch is given off which extends upwards at the sides of the stomodæum and here causes a slight protrusion of the ectoderm, so that the outline of the anterior part of the larva becomes quadrate instead of being rounded as it was pre-

viously. This extra rod is the "antero-lateral rod" of the larval skeleton and the protrusion is the rudiment of the antero-lateral arm. Meanwhile the post-oral rod has pushed out a posterior protrusion of the ectoderm on each side just above the blastopore, now become the anus. This protrusion is the rudiment of the post-oral arm (called "anal arm" by Driesch and Herbst). Up till now the larva has been uniformly ciliated all over, with a tuft of specially long cilia at the anterior pole, but now the cilia become restricted to a ridge forming the edge of the concave ventral surface. This is the longitudinal ciliated band of the larva, and it runs along the edges of the protrusions which are the rudiments of the larval arms.

The tuft of specially long cilia becomes incorporated in the anterior border of this band.

A dorsal view of a larva in this stage is given in Pl. 14, fig. 4. As soon as the alimentary canal is complete spots of orange-red pigment appear just under the ectoderm all over the larva. The pigment is carried by wandering mesenchyme cells, which can be seen to migrate into the ectoderm and to discharge their pigment (which is probably of an excretory nature) to the exterior in the form of granules. During the next day the antero-lateral and post-oral arms grow longer and an adoral band of cilia (*ad.*, fig. 5) becomes well defined. This consists of two ridges of thickened epithelium lying in the ventro-lateral walls of the larval œsophagus belonging partly to the ectodermal and partly to the endodermal region of this, which carry long cilia. There is reason to believe that these cilia produce an outwardly directed current and that their function is to remove excess of food matter from the region of the mouth. At least if a living larva be watched, particles suspended in the water shoot out violently from the ventral side of the mouth. On the left side the madreporic pore-canal and pore are formed by the union of a dorsally directed up-growth from the left cœlomic sac and a slight in-pitting of the ectoderm. Constrictor muscles, which cause the œsophagus to exe-

cute peristaltic swallowing movements, are developed from the inner walls of the cœlomic sacs, where they rest against the œsophagus; and dilator muscles are represented by protoplasmic strings (*dil.*, Pl. 14, fig. 5) which join the antero-lateral rods to the outer walls of the cœlomic sacs. A larva four days old viewed from the dorsal side is shown in fig. 5. When the larva has attained the age of six days it has increased greatly in size, and rudiments of the remaining four arms, viz. the two præ-oral and the two postero-dorsal, are visible as very slight protrusions of the ciliated band. Underneath the rudiments of the postero-dorsal arms is seen an accumulation of mesenchyme cells, in the centre of which a high power of the microscope reveals a minute calcareous spicule—the rudiment of the skeleton of the arm.

No such accumulation is seen beneath the rudiments of the præ-oral arms; their skeleton arises in a later stage, as a median dorsal spicule, situated above the œsophagus far from the arms, and the actual præ-oral rods are subsequent outgrowths from this spicule. This is important, because it proves that the outgrowth of the arm is not directly due to a mechanical push exercised by the growing arm rod, but must rather be due to a chemical influence emanating from the spicule. The aboral ends of the body rods become bent inwards in a crook-like form (*cr.*, fig. 6), often far better shown than in the specimen figured.

## (2) THE DEVELOPMENT OF ECHINOCARDIUM CORDATUM.

The egg of *Echinocardium cordatum* is not more than two thirds the diameter of the egg of *Echinus esculentus*, and the blastula which develops from it and rises to the top about eighteen hours after fertilisation is correspondingly small. Moreover, it is not spherical, but is elongated along one axis more than along the other. It is not, however, regularly oval, but would be more correctly described as being of cylindrical shape, rounded at the ends. In fig. 7 (Pl. 14) one of these blastulæ is shown. It is a little older than the blastula of *Echinus* shown in fig. 2, and the mesenchyme is more

developed. The blastula in course of the next day becomes a gastrula, and this stage is shown in fig. 8. In the gastrula represented in this figure we see that the mesenchyme is arranged in a circle; it is termed the primary mesenchyme, because it is given off as in the Echinus embryo from the vegetative pole of the blastula before the invagination which forms the archenteron has begun. At two opposite points in this circle a special aggregation of mesenchyme is to be seen, and inside each aggregation there is already clearly to be made out the four-armed calcareous "star" which is the rudiment of the larval skeleton. Of the four arms, one is directed upwards towards the anterior pole of the larva; this becomes, as in Echinus, the antero-lateral rod. One is directed backwards towards the blastopore; this becomes the "body rod." One is directed horizontally and forms the horizontal rod; whilst the fourth is directed outwards into a very slight elevation as ectoderm. This elevation of the ectoderm is the first trace of the post-oral arm, and the arm of the star corresponding to it is the rudiment of the post-oral rod. This "rod" is double, i.e. it is represented by two rods parallel to each other. At the anterior pole of the larva is to be seen the group of specially long cells carrying specially powerful cilia (*cil.*) similar to what was described in the case of the larva of Echinus. The secondary mesenchyme, which is budded from the apex of the archenteron and becomes the loose connective tissue of the larva, is well seen in the figure. The gastrula rapidly develops into an Echinopluteus larva by the same stages as those described in the case of Echinus. A concave ventral surface becomes defined, and to the edges of this surface the cilia become confined. The anterior tuft of cilia becomes incorporated in the anterior border of this surface, and the post-oral arms also arise from its border. The stomodæum arises as a pit on the ventral surface, and in the larva shown in fig. 9 this is just touching the anterior apex of the archenteron, although the two cavities are not yet open into each other. The coelom arises, as in Echinus, as a transversely

elongated outgrowth from the archenteron, which becomes nipped off and then divided into right and left vesicles. Spots of dark red pigment have been developed. But it is the skeleton which especially arrests our attention. The post-oral arms are already developed, and each is supported by two parallel rods connected with each other by numerous cross-bars. The antero-lateral arms have not yet appeared, but the antero-lateral rods are already formed—each a single calcareous rod. From each of these rods a branch (*r. r.*) projects backwards, this is the rudiment of the recurrent rod. The body rods are well-developed single bars. Between their aboral ends an accumulation of mesenchyme cells can be seen, in the centre of which a tiny calcareous star (*ab*) can be seen. This accumulation of cells represents the formative matrix of the skeleton of the aboral spike—a structure characteristic of the Spatangoid larva and not found in the larva of any regular Echinoid. During the course of the next day the antero-lateral arms sprout out and so does the club-shaped aboral spike. The recurrent rod has grown backwards parallel to the body rod which it now equals in length; this rod is usually vestigial in *Echinus esculentus*, but is shown on one side in the larva represented in fig. 6. But in *Echinocardium cordatum* the recurrent rods extend to the aboral pole and here fork; the dorsal fork unites with its fellow, whilst the ventral forks unite the ends of the body rods, and in this way a terminal ring is formed in every way comparable to the so-called “frame” at the aboral end of the *Sphærechinus* larva. The skeleton of the aboral spike (*ab*) consists of three slightly diverging rods connected with each other by cross-bars. At their anterior ends they join the terminal ring, which has just been described. One of these rods is dorsal and median, the other two are lateral. It follows that the aboral spike possesses a most complicated skeleton. The apex of the aboral spike is covered with a crest of long-ciliated cells (*cil.*, figs. 10 and 11). During the course of the next day the rudiments of the postero-dorsal arms make their appearance and in each there is a tiny star,

the rudiment of its skeleton (*p.d.*). The coelomic sacs, which in the previous stage lay at the sides of the œsophagus, now begin to grow back along the sides of the stomach and on each side a madreporic pore is formed. Whether this duplicity of the madreporic pore is constant or not I have not yet been able to determine; it is, at any rate, very frequent. A larva in this stage of development is represented in fig. 11. Further than this stage it is not my intention to pursue the history of the development. The larva of *Echinocardium* has in four days reached the same stage of development as that attained by the larva of *Echinus esculentus* in six days.

Let us now review the differences between the two types of larvæ. Leaving out the question of the duplicity of the madreporic pore, which may turn out to be a most important character but which cannot be used at present—we find that the larva of *Echinocardium* differs from that of *Echinus*—

(1) In possessing an aboral spike supported by a complicated skeleton, whilst at the aboral pole of the *Echinus* nothing of this kind is to be seen.

(2) In possessing “latticed” bars instead of single rods as supports for the post-oral arms. (Exceptionally in *Echinus* a second rod can appear in the post-oral arm. A trace of such rod is represented in fig. 4. I have never seen cross-connections between the two, but I am informed that these also can appear as a rare exception.)

(3) In possessing well-developed recurrent rods instead of only vestiges of such rods.

(4) In possessing brownish-red instead of orange-red pigment.

(5) In its more rapid development.

We shall now see how far these characters are represented in the hybrids.

#### DEVELOPMENT OF EGGS OF *ECHINOCARDIUM CORDATUM* FERTILISED WITH THE SPERM OF *ECHINUS ESCULENTUS*.

The earlier course of the development of *Echinocardium* eggs fertilised with the sperm of *Echinus*

*esculentus* is very similar to that undergone by these eggs when fertilised with the sperm of their own species. Fig. 12 shows the appearance of the free-swimming blastula twenty-four hours fertilisation. In this specimen the vegetative end is rather broader than the animal end, whereas in the blastula of *Echinocardium* represented in fig. 7, the reverse is the case, but this difference between the hybrid and normal blastula is not constant. By the end of the second day the blastula is converted into a gastrula such as is shown in fig. 13. The rate of development of the hybrid, however, varies with the specimens used as parents. In fig. 14 a hybrid larva of the same age is represented, which, however, belonged to a different culture, and we can see that it has attained the stage where the calcareous stars have been formed and where the coelom is already grooved off from the archenteron. If from the same specimen eggs are taken some of which are fertilised with sperm of its own species and some with the sperm of *Echinus esculentus*, then the hybrids will always develop more slowly than the normal larvæ. As development proceeds the hybrids fall more and more behind the normal larvæ. In fig. 15 a hybrid four days old is represented. In this specimen the post-oral arms are well developed but the stomodæum has not joined the œsophagus. The bars supporting these arms are "latticed," but the cross bars are comparatively few. The antero-lateral bars are there but the antero-lateral arms are not yet developed. As shown in fig. 16, however, hybrids four days old may be more advanced in development. In the specimen shown in this figure the alimentary canal is complete and the post-oral arms are longer than in the specimen shown in fig. 15. But the supporting bars of these arms are single rods for the greater part of their length, although a second short rod accompanies the first at its base, as it does as a variation in *Echinus esculentus*. Both larvæ agree in the total absence of any indication of the aboral spike or of its skeleton.

When we turn our attention to the hybrid larvæ five days



old which are shown in figs. 17 and 18 the same total absence of an aboral spike or any indication of its skeleton strikes us. The antero-lateral arms are now developed. In the larva represented in fig. 17 the skeleton of one post-oral arm consists at its base of no less than four parallel rods; more distally a curious vestige of latticing is seen in the form of short rods accompanying the main rod and each connected with it by a transverse bar. The ends of the body rods are in-bent in a crook-like fashion such as occurs in the normal larva of *Echinus esculentus*. The larvæ represented in fig. 18 has an almost normal *Echinocardium* skeleton in each of the post-oral arms, but it is absolutely devoid of the aboral spike. In fig. 19 a hybrid six days old is represented; it shows much the same features as those shown by the five-day hybrid represented in fig. 18, only the arms are better developed and the crook-like in-bending of the aboral ends of the body rods is very marked. We notice also that a recurrent rod is well developed. In the seven-day larva shown in fig. 20 one of the antero-lateral arms has been absorbed—a phenomenon which often occurs with normal larvæ which are not quite healthy. The skeleton of this larva shows hardly any trace of the maternal influence; it is almost purely of the paternal type.

None of the hybrids lived longer than eight days although they were supplied with abundant food. Three of these eight-day larvæ are represented in figs. 21, 22 and 23. That shown in fig. 21 has a skeleton almost purely paternal in character; the only hint of maternal influence is to be seen in the thorns besetting the single rods which constitute the skeleton of the post-oral arms. One striking maternal character is, however, shown in the duplicity of the madreporic pore. The larva shown in fig. 22 has a double rod in each post-oral arm, but on one side the two rods are fused into one for the middle of their length, whilst on the other they are widely separated from one another and connected by a few cross-bars. Finally in the remarkable larva shown in fig. 23 there is a skeleton of a purely maternal type, and the aboral spike

is typically developed. The larva is distorted, however, since the oral lobe containing stomodæum is, as it were, twisted round on the body, and the result of this twist on the disposition of the skeletal rods is at first not a little puzzling. Care, however, enables us to recognise all the constituent parts of the maternal skeleton.

To sum up: The hybrid produced by fertilising the eggs of *Echinocardium cordatum* with the sperm of *Echinus esculentus* follows the mother in the character and distribution of the pigment: it is much smaller than larvæ of either the paternal or maternal species; it almost always follows the father in the total absence of the aboral spike and of its supporting skeleton, since in only one hybrid out of the hundreds examined was the aboral spike formed. In the skeleton of the post-oral arms the hybrid may be of the paternal type, of the maternal type, or of an intermediate character. In the inbending of the aboral ends of the body rods the hybrid follows the father.

The most important of these results is undoubtedly the total inhibition in the vast majority of cases of the formation of the aboral spike in a larva developed from a *Spatangid* egg, and the formation of a larva with a rounded aboral end and in-bent body rods, recalling in these features the *Echinus* larva. When we recollect that according to Shearer, De Morgan and Fuchs the crossing of two species of the genus *Echinus* results in the production of larvæ of the maternal type, no matter what feature is considered, it is not a little remarkable to find the paternal influence so strong in a cross between two species belonging not only to different genera but to different orders, species which must have diverged from a common ancestor at the beginning of the secondary epoch many millions of years ago.

If the attempt be made to explain the absence of the aboral spike as a mere concomitant of the feeble development of the hybrid, a glance at the figures of normal *Echinocardium* larvæ will be sufficient to refute this suggestion. We see there that the aboral skeleton and its formative

mass of mesenchyme can be detected in the normal larva before the antero-lateral arms have developed at all, and in many of the hybrids the antero-lateral arms are well developed. The absence of the aboral spike is therefore not a mere consequence of stunted growth, but is due to paternal influence. Moreover, it is not possible to reconcile the facts just recorded with any theory of dominance such as that put forward by Loeb, Redman, King and Moore.

If any clear meaning be attached to the word "dominance," it must signify that there is a certain factor which may be present or absent in a germ-cell, but which, when it is present, produces in the resulting embryo a certain character. Now if we take the question of the skeletal rods supporting the aboral arms, these are normally single in *Echinus* and latticed in *Echinocardium*. When these two species are crossed we do not always find either single or latticed bars, but we find, as a matter of fact, every intermediate condition of affairs. To alter the significance of the word "dominance" in such a way as to make it include phenomena like these is, in my opinion, to empty it of all its meaning.

ZOOLOGICAL LABORATORY,

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY;

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### EXPLANATION OF PLATES 14 AND 15,

Illustrating Prof. E. W. MacBride's paper entitled "Studies on the Development of Echinoidea, Part II."

#### LIST OF ABBREVIATIONS EMPLOYED.

*ab.* Skeleton of aboral spike of Echinocardium larva. *ad.* Adoral ciliated band. *a.l.* Skeleton of antero-lateral arm of larva. *an.* Anus. *b.r.* Body-rod of skeleton of larva. *calc.* First rudiment of skeleton of larva. *cil.* Anterior tuft of long cilia and posterior tuft of long cilia in Echinocardium larva. *cæ.* Cœlomic sac. *cr.* In-bent crook of body-rod in skeleton of larva of hybrid and of Echinus. *dil.* Dilator muscles of cœlomic sac. *f.* Fertilisation membrane. *h.r.* Horizontal rod of larval skeleton. *int.* Intestine. *l.p.c.* Left posterior cœlom. *mad.* Normal left madreporic pore. *mad.*<sup>1</sup> Right madreporic pore of Echinocardium larva. *mes.* Primary mesenchyme. *æs.* Œsophagus. *p.d.* Rudiment of skeleton of postero-dorsal arm. *pr.o.* Rudiment of præ-oral arm. *p.o.* Skeleton of post-oral arm. *r.p.c.* Right posterior cœlom. *st.* Stomach. *stom.* Stomodæum.

[All the figures were drawn from living larvæ with the help of the camera lucida, and all the larvæ figured are represented with a uniform magnification of 200 diameters so that their relative sizes can be seen.]

#### PLATE 14.

Fig. 1.—Egg of Echinus esculentus treated with the sperm of Echinocardium cordatum and examined twenty-four hours afterwards. *f.* Fertilisation membrane.

Fig. 2.—Blastula of Echinus esculentus twenty hours old. *mes.* Primary mesenchyme in the act of being formed.

Fig. 3.—Late gastrula of Echinus esculentus two and a half

days old. *cæ.* Cœlomic sac just separating from archenteron. *b. r.* Body-rod. *h. r.* Horizontal rod, and *p. o.* post-oral rod as three branches of trifold calcareous star, the rudiment of the larval skeleton.

Fig. 4.—Larva of *Echinus esculentus* three days old viewed from the dorsal side. *a. l.* antero-lateral rod of skeleton. *cæ.* Cœlomic sacs. *æs.* Endodermal part of œsophagus. *stom.* Stomodæum. *p. o.* Post-oral rod of skeleton (notice the supplementary rod on right side).

Fig. 5.—Larva of *Echinus esculentus* four days old viewed from the dorsal surface. *ad.* Thickening of epithelium carrying adoral ciliated band. *dil.* Dilator muscles of cœlomic sac and of œsophagus attaching these structures to the antero-lateral rod of the larval skeleton. *mad.* Madreporic pore.

Fig. 6.—Larva of *Echinus esculentus* six days old viewed from the dorsal surface. *cr.* Inbent crook at aboral end of body-rod. *p. d.* Accumulation of mesenchyme cells, the formative tissue of skeleton of postero-dorsal arm. *p. r. o.* Rudiment of præ-oral arm. *r. r.* Vestige of recurrent rod of skeleton.

Fig. 7.—Blastula of *Echinocardium cordatum* twenty-four hours after fertilisation. *mes.* Primary mesenchyme being formed.

Fig. 8.—Gastrula of *Echinocardium cordatum* one and a half days old. *calc.* Rudiment of skeleton. *cil.* Anterior tuft of long cilia. *mes.* Secondary mesenchyme being budded from the apex of the archenteron.

Fig. 9.—Larva of *Echinocardium cordatum* two days old viewed from the dorsal surface. *ab.* Calcareous star, the rudiment of the skeleton of the aboral spike embedded in a mass of formative mesenchyme. *p. o.* Latticed skeleton of post-oral arm. *æs.* Endodermal œsophagus which is in contact with, but which has not yet opened into, *stom.* the stomodæum. *r. r.* Recurrent rod of the skeleton.

Fig. 10.—Larva of *Echinocardium cordatum* three days old viewed from the dorsal surface. The antero-lateral arms have grown out and the stomodæum has opened into the œsophagus. The aboral spike and its skeleton are fully formed. *cil.* Posterior tuft of cilia at the apex of the aboral spike.

Fig. 11.—Larva of *Echinocardium cordatum* four days old, viewed from the dorsal surface. *l. p. c.* Backward growth of the cœlom on the left side of the stomach, which will be cut off as the left posterior cœlom. *r. p. c.* Similar growth of the cœlom on the right side of the stomach which will be cut off as the right posterior cœlom. *mad.* Left madreporic pore. *mad.* Right madreporic pore. *p. d.* Rudiment of the skeleton of the postero-dorsal arm.

## PLATE 15.

[All the following figures represent larvæ developing from the eggs of *Echinocardium cordatum* which have been fertilised with the sperm of *Echinus esculentus*.]

Fig. 12.—Hybrid blastula twenty-four hours old. *mes*. Primary mesenchyme.

Fig. 13.—Hybrid gastrula two days old. *cil*. Anterior tuft of cilia:

Fig. 14.—Hybrid gastrula (from another culture) two days old. *cæ*. Rudiment of cælom. *calc*. Rudiment of skeleton. *mes*. Secondary mesenchyme.

Fig. 15.—Hybrid larva four days old viewed from the dorsal side. *a.l*. Antero-lateral rod of skeleton. *b.r*. Body-rod. *cil*. Anterior tuft of cilia now incorporated with longitudinal ciliated band. *æs*. Larval œsophagus not yet joined to stomodæum. *r.r*. Vestigial recurrent rod.

Fig. 16.—Hybrid larva four days old, more advanced in development than that shown in fig. 15; viewed from the dorsal side.

Fig. 17.—Hybrid larva five days old viewed from the dorsal surface. *c.r*. In-bent crook at aboral end of body-rod.

Fig. 18.—Hybrid larva five days old viewed from the left side. *ad*. Ridge of thickened epithelium in the œsophagus carrying the aboral ciliated band. *an*. anus. *h.r*. Horizontal branch of the skeleton.

Fig. 19.—Hybrid larva six days old viewed from the dorsal surface. *r.r*. Recurrent rod.

Fig. 20.—Hybrid larva seven days old viewed from the dorsal surface. Notice that one antero-lateral arm has been absorbed.

Fig. 21.—Hybrid larva eight days old viewed from the dorsal surface. *mad.*, *mad.*<sup>1</sup> The two madreporic pores.

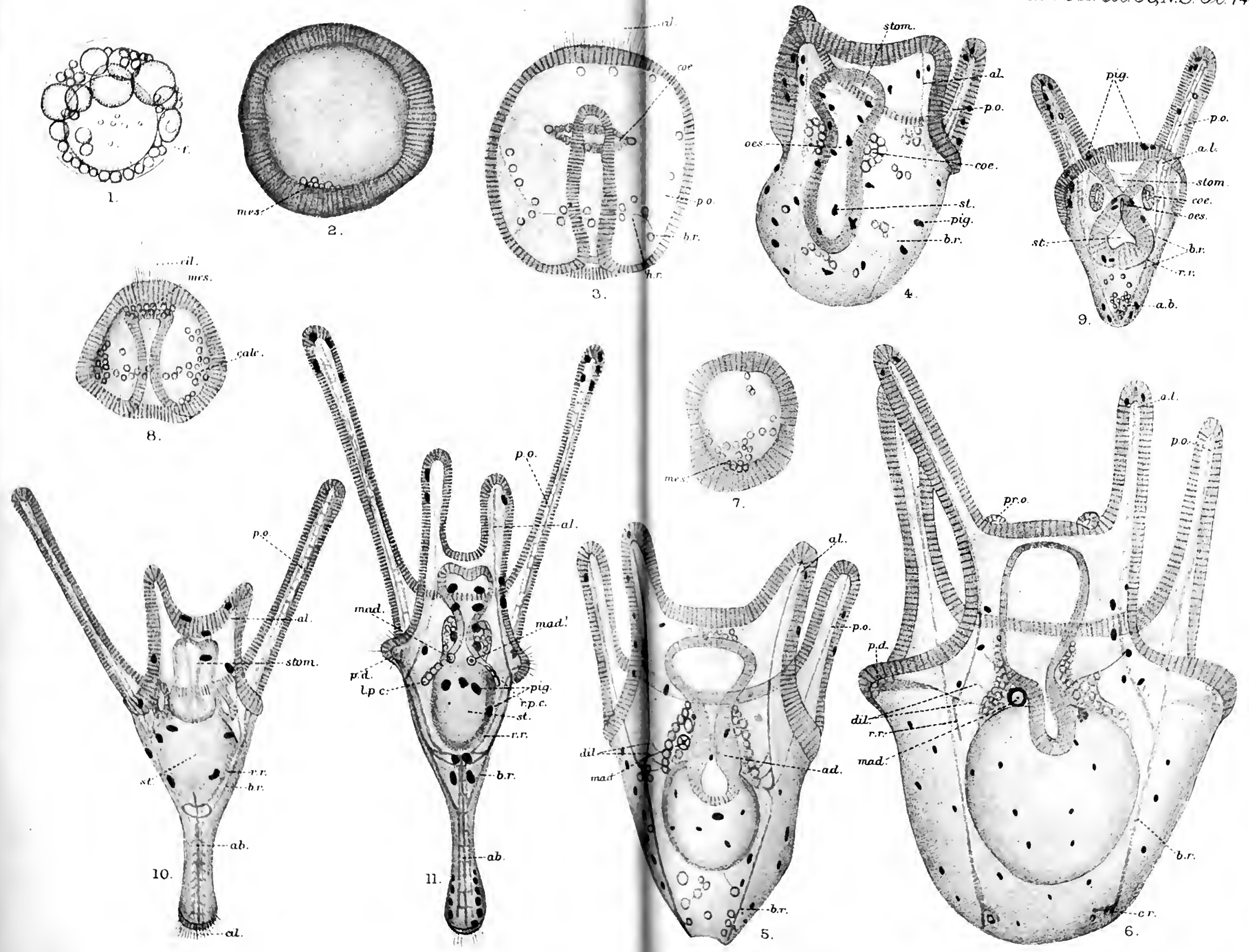
Fig. 22.—Hybrid larva eight days old viewed from the ventral surface. *an*. Anus. *h.r*. Horizontal branch of larval skeleton.

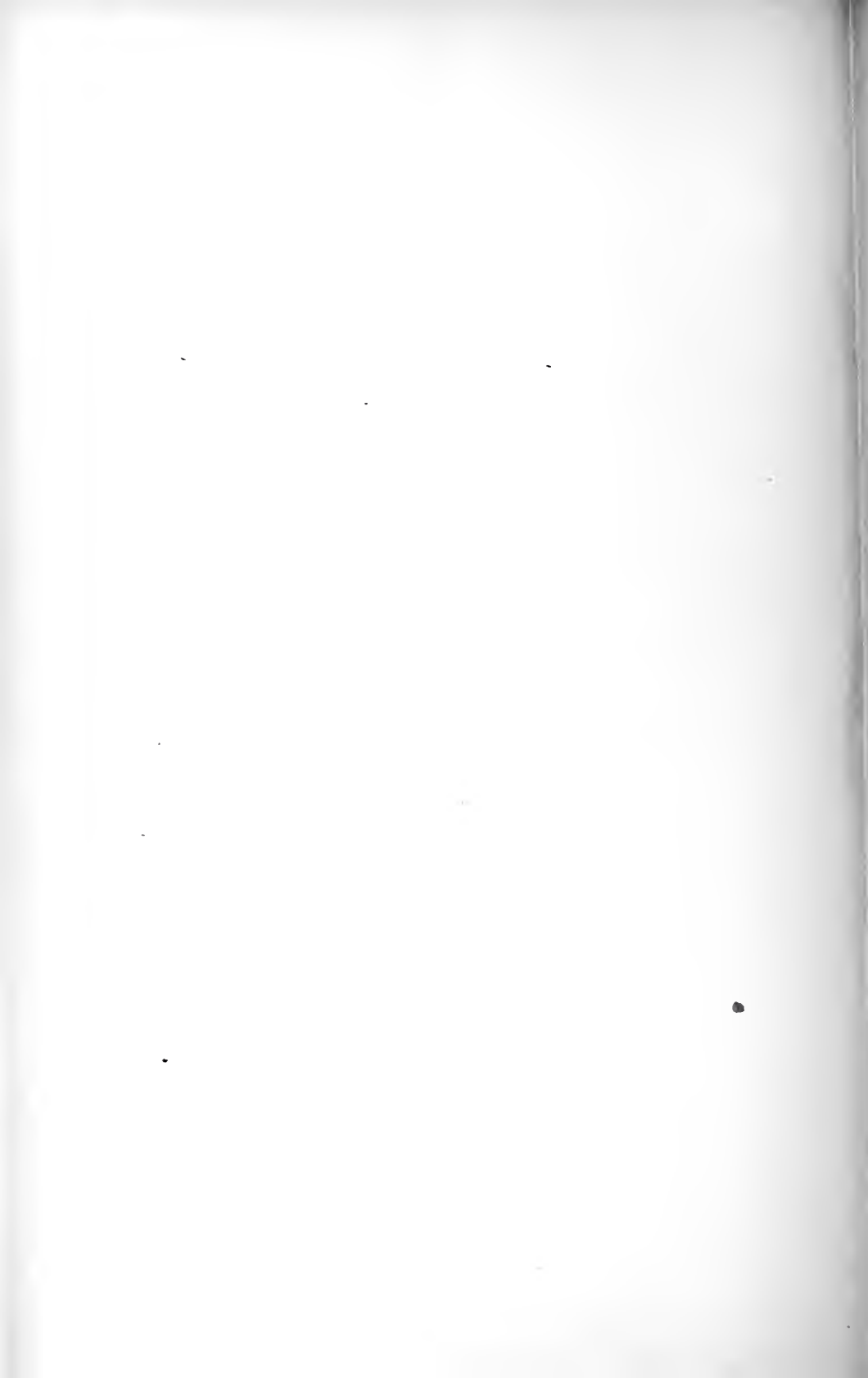
Fig. 23.—Hybrid larva eight days old viewed from the left side. This is the only hybrid in which the aboral spike and its skeleton have been developed. Notice the distortion of the larva. It is as if the left antero-lateral arm and the left side of the oral lobe had been forcibly twisted away from the spectator. *b.r*. The right and left body-rods crossing each other. *cil*. Posterior tuft of cilia.







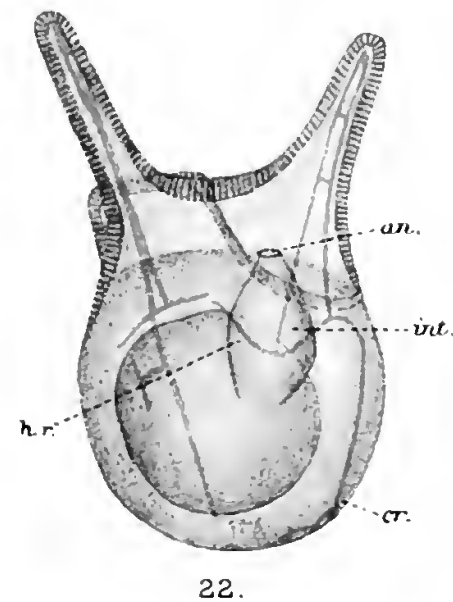
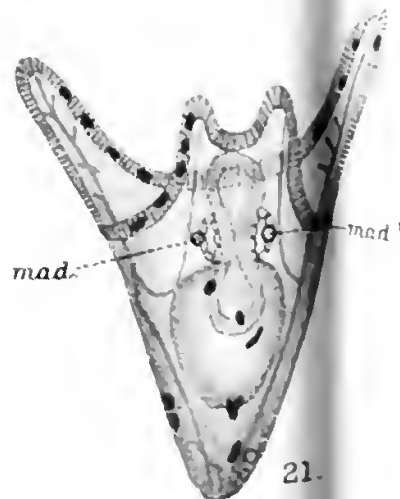
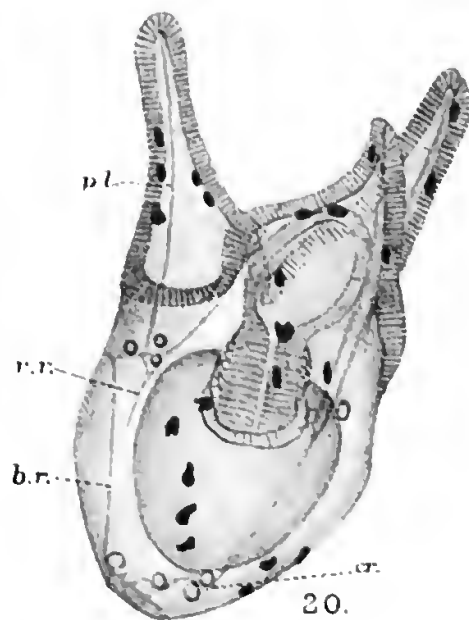
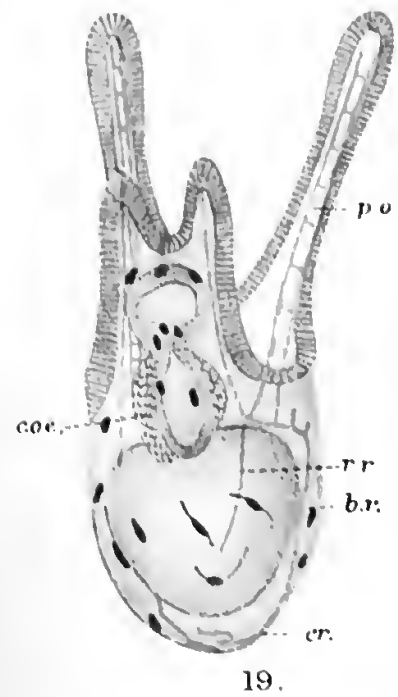
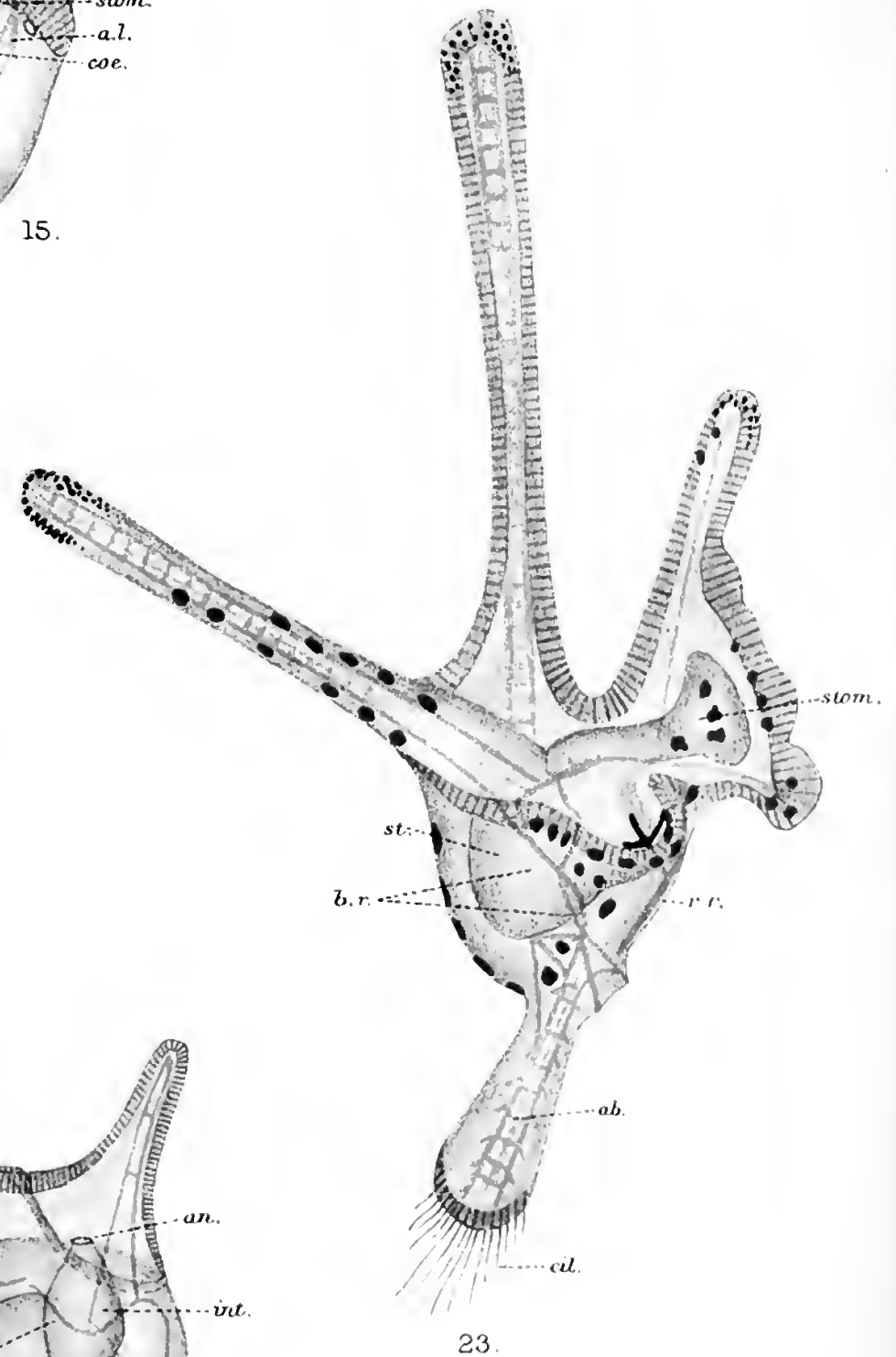
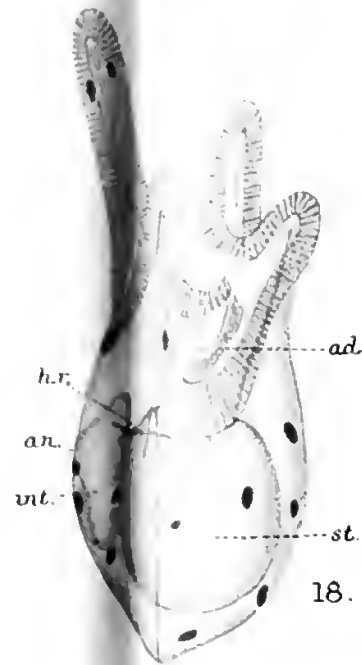
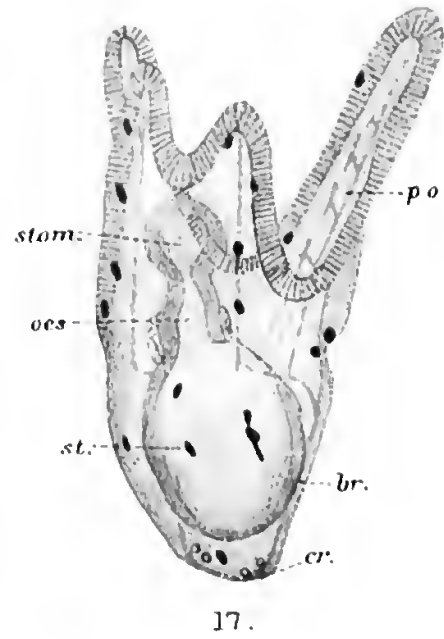
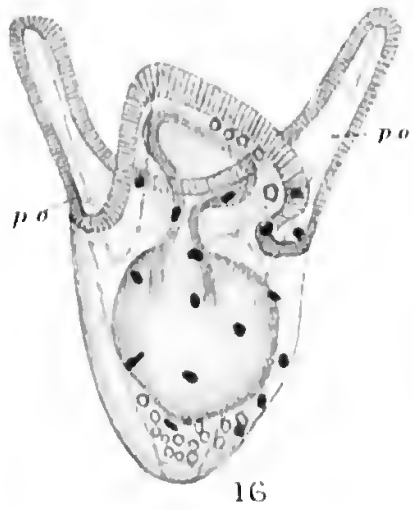
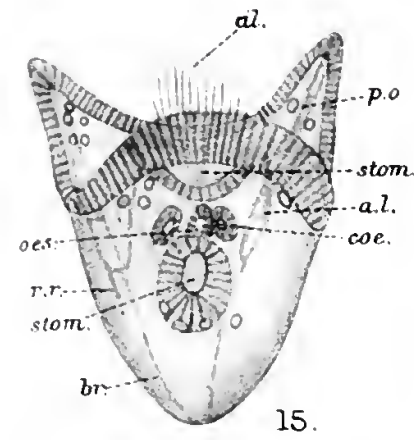
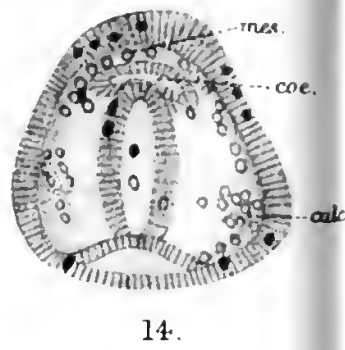
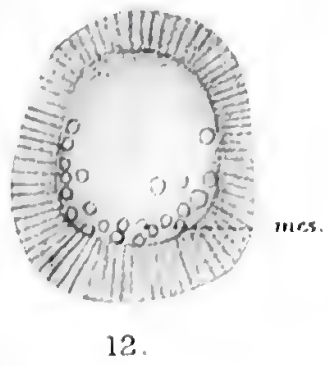




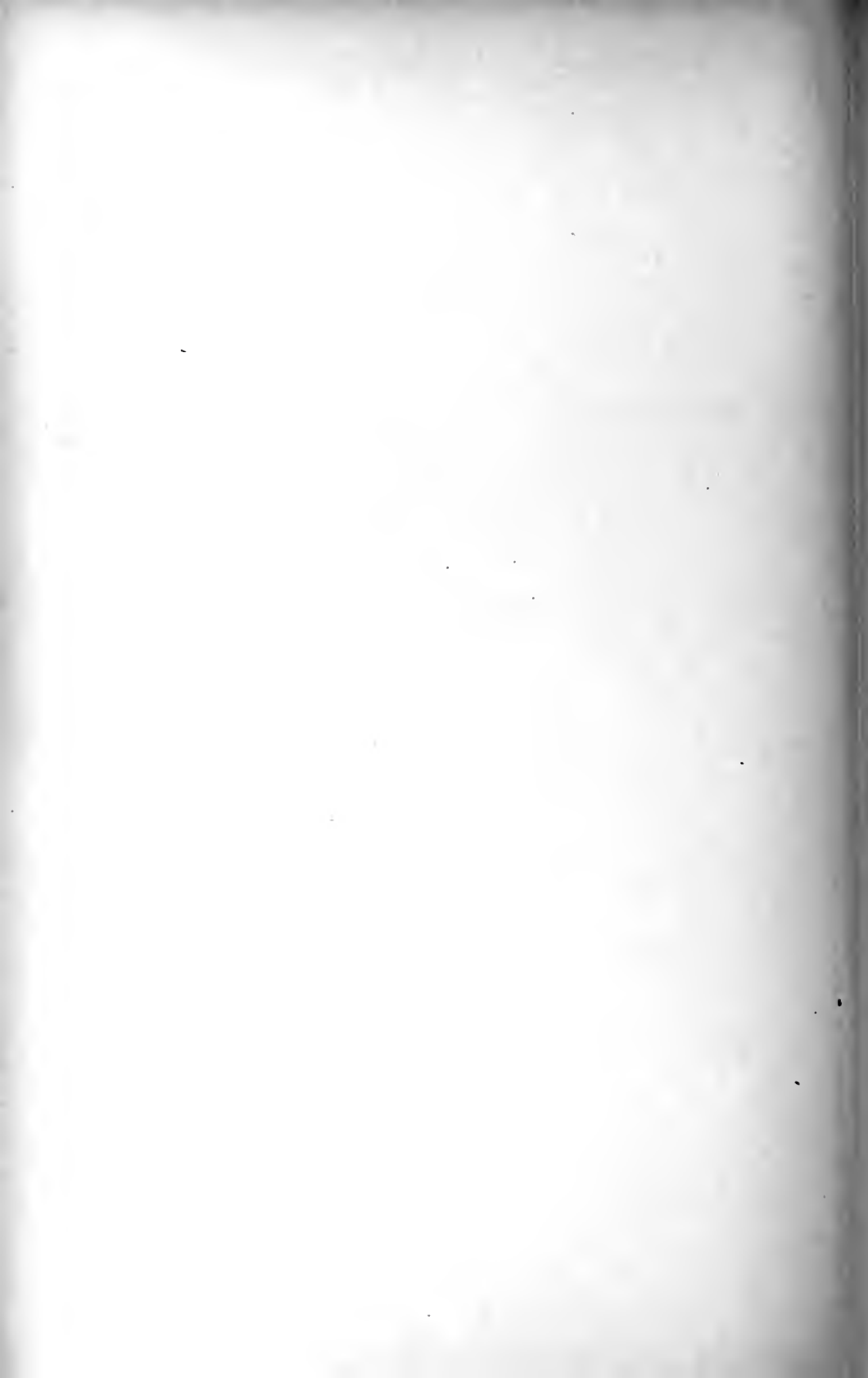












**The Experimental Hybridisation of *Echinus miliaris*, *Echinus esculentus*, and *Echinus acutus*.**

By

**G. Debaisieux,**

Assistant in Zoology at the University of Louvain (Belgium).

From the Imperial College of Science and Technology, London.

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With Plate 16.

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THE few experiments on the hybridisation of the Echinoderms of which I am now giving the results were commenced on the advice of Professor MacBride, who himself had made similar experiments on other species. At his suggestion I chose *Echinus miliaris*, *Echinus esculentus* and *Echinus acutus*, in order to verify the interesting results obtained by Shearer, De Morgan and Fuchs ('11) in their experiments on these species. I should like in the first place to express my thanks to Professor Sedgwick, who so kindly received me at his institute, and to Professor MacBride, who has helped me so much by his valuable advice and has placed at my disposal material which he had himself collected.

The very numerous researches which have already been made in the hybridisation of the Echinoderm of various species have given results which appear to be very varied. Boveri ('89 and '95) in his crossings of *Sphærechinus* and *Echinus*, remarks that the characters of hybrids are mixed and inter-

mediate between the paternal and the maternal. These conclusions were soon considered doubtful by Seeliger ('94) and Morgan ('95), and very numerous experiments were again made on different material. In nearly all these experiments the student started with the idea of finding out if the hybrids had characters which were exclusively maternal, exclusively paternal, or intermediate between the characters of the two parents.

Driesch ('98) studied the crossings between *Sphærechinus*, *Strongylocentrotus*, *Echinus* and *Arbacia*, and he remarked that "Während alle anderen untersuchten Charaktere der Bastardlarven von Echiniden sich als rein mütterlich und damit sehr wahrscheinlich als von der Natur des Eiplasmas abhängig erweisen, treten bei der Skelettbildung zuerst die Charaktere der väterlichen species auf, indem sich dieselben als eine Mischung väterlicher und mütterlicher Eigenschaften, je nach der kombination mehr zum vater (*Sph.* ♀ × *Echinus* ♂, *Sph.* ♀ × *Str.* ♂) oder mehr nach der Mutter (*A.* ♀ × *E.* ♂, *E.* ♀ × *A.* ♂) hinneigend darstellt." The same author, in a more recent work ('03), does not notice the transmission of pigmentary characteristics by the father, but relying on Boveri's work, he considers that this transmission is sometimes possible, whereas Fischel's experiments and conclusions ('06) prove a certain paternal influence and the heredity of the paternal characters. Other material was used by Vernon ('98). He used eggs of *Echinocardium cordatum* and fertilised them by sperm of *Echinus*, *Strongylocentrotus*, *Sphærechinus* or *Arbacia*. The hybrids were all of the maternal type, but the aboral spike was considerably shorter in them than in the normal larvæ. In a case where the opposite crossing was successful, *Echinus* ♀ × *Echinocardium* ♂, the characters of the hybrids were exclusively maternal. The same material was again experimented on by MacBride ('11) and the results were quite different: whilst the crossing of *Echinus* ♀ × *Echinocardium* ♂ did not give larvæ, the hybrids *Echinocardium* ♀ × *Echinus* ♂ presented paternal and maternal characters.

In another set of experiments on *Sphærechinus* and *Strongylocentrotus*, Vernon ('00) notices considerable variations in the hybrids which he obtained.

At times they present characters which are almost exclusively maternal, at others almost exclusively paternal, and again, at times, characters more or less approaching one or the other. These results are confirmed by Steinbrück ('02), by Doncaster ('04), and by Herbst ('06 and '07), but whilst Vernon attributes these variations to the influence of the seasons, the last authors, above quoted, are more precise, and attribute them to the changes in the temperature.

Equally contradictory results were given by the crossings of other species. Hagedoorn ('09) studied only one characteristic of the skeleton of the hybrids of *Strongylocentrotus franciscanus* and *Strongylocentrotus purpuratus*. He discovered a purely maternal heredity; on the other hand, Loeb, King and Moore ('10) and Moore ('12), taking up the same study notice that the different characters of the skeleton of the hybrids are not of maternal origin, but always follow the law of dominance, that is to say that the same character is always present or absent in the hybrid, whether it is  $F. \text{♀} \times P. \text{♂}$  or  $P. \text{♀} \times F. \text{♂}$ .

Very similar conclusions were drawn by Tennent ('10) in his experiments in crossing *Hipponoë* and *Toxopneustes*, but he remarks that the characters which follow the law of dominance vary according to the alkalinity of the water which is used in the experiments.

At last we come to the experiments of Shearer, De Morgan, and Fuchs ('11). After examining the hybrids of *Echinus miliaris*, *esculentus* and *acutus*, the authors conclude:

A. "There is considerable evidence for the contention put forward by Loeb, King and Moore ('10) that the minor skeletal characters are inherited independently from either parent.

B. "In the presence or absence of the posterior ciliated epaulettes, of the green pigment masses, and of the posterior pedicellaria, we claim that we have found definite characters,

and we find them to be invariably inherited through the egg."<sup>1</sup>

It is to verify the accuracy of this last opinion that I have again taken up the experiments of hybridisation of *Echinus miliaris*, *esculentus* and *acutus*. The animals which we used were collected at Plymouth and sent to London, where they always arrive perfectly fresh and in a fit state to give good fecundation. I used methods which were advised by Professor MacBride, and which he himself has described in two articles ('03, '11). I have only to add that the most minute precautions were taken to avoid errors of any description. The animals were carefully washed in fresh water before being dissected; all the instruments and utensils were sterilised before use; sea-water was never used unless it had been kept for a long time in the laboratory and filtered through a Berkfeld filter.

I principally concentrated my attention on three characters which are very characteristic of the species and easy of observation.

(i) The posterior epaulettes which are present in *Echinus esculentus* and *acutus*, absent in *Echinus miliaris*.

(ii) The posterior pedicellaria which is present in *Echinus esculentus* and *acutus*, absent in *Echinus miliaris*.

(iii) The green pigment which is absent in *Echinus esculentus* and *acutus*, present in *Echinus miliaris*.

It is noticeable that, starting with these three characters, it is impossible to distinguish the larvæ of *Echinus esculentus* and of *Echinus acutus*. Besides, no differential character has been recorded in these larvæ. The hypothesis that they are two varieties of the same specie becomes more and more probable. Besides, the results which I obtained were identical whether I used *E. esculentus* or *E. acutus*.

<sup>1</sup> A letter from Shearer, De Morgan and Fuchs, which appeared in a recent number of 'Nature,' June 27th, 1912, states that this year's experiments give results which are quite different from those of other years—results which are doubtless in a great measure analogous to those which we have obtained. I shall discuss their opinion regarding this variation farther on.

I obtained the pure larvæ for each type (figs. 1, 2, 3) and the four hybrids which are possible :

M. ♀ × E. ♂ (fig. 4) ; E. ♀ × M. ♂ (figs. 5, 6) ; M. ♀ × A. ♂ (figs. 7, 8) ; A. ♀ × M. ♂ (figs. 9, 10).

I was able to rear all the hybrids of *E. acutus* through the metamorphosis until they reached the stage of the adult echinus. The hybrids of *E. esculentus* did not go beyond the metamorphosis.

The heredity of the three characters which I studied was as follows :

(1) The posterior epaulettes were present in all the larvæ of the four kinds of hybrids.

This character, therefore, was transmitted to the bastard larvæ in each case, in some through the father, in others through the mother. This character, however, is not wholly transmitted to hybrids. In the larvæ of pure *E. acutus* or *esculentus* the epaulettes are formed at the expense of the loop of the ciliated band which intervenes between the post-oral arm and the postero-dorsal arm, and then the epaulettes entirely separate themselves from it. Now in hybrids we have observed that the posterior epaulettes remain attached to this commissure, and do not separate themselves from it. Consequently the epaulettes are not so perfectly formed in hybrid larvæ as in pure larvæ.

(2) The posterior pedicellaria is to be found in the following hybrids: M. ♀ × A. ♂, A. ♀ × M. ♂, E. ♀ × M. ♂, and probably also in M. ♀ × E. ♂. But the posterior pedicellaria is sometimes absent in certain individual larvæ. One must notice that in the pure larvæ of *E. miliaris*, one does not find a pedicellaria at the posterior pole, but a cellular proliferation in which a calcareous plate is formed which often bears a spine. Now in hybrids one sometimes finds one spine, sometimes two, and sometimes two spines and a pedicellaria.

(3) The green pigment is never transmitted to the larvæ in any of the four kinds of hybrids. This green pigment is present in the larvæ of *Echinus miliaris* in four

little masses which are in the concavity of the four anterior epaulettes, and it is scattered in little grains in the arms and along the ciliated band. In the hybrid larvæ it is not to be found in any of these places. One might suppose that the disappearance of the green pigment is due to degeneration or to a weakness of the hybrids. In fact it is noticeable that the pigment diminishes considerably in the larvæ of *E. miliaris* when in a weak state, as, for instance, when they are hungry. This supposition cannot be maintained if one considers (1) that the green pigment never completely disappears in the pure larvæ, (2) that this diminution is accompanied by the diminution of the red pigment. Now in hybrids green pigment is always entirely absent and the quantity of red pigment is not lessened; rather the contrary.

To sum up and to explain these results it seems necessary to look at the question from another point of view than that which is customary.

In these experiments of crossing amongst *Echinus miliaris*, *esculentus*, and *acutus*, there is not exclusively maternal or paternal heredity; one might say that the hybrids inherit simultaneously from the paternal and the maternal influence; but this formula does not express all the results obtained, for there is no mention of the fact that certain characters are always absent or always present in all hybrids, and that the paternal or maternal origin of the characters does not influence their transmission to descendants.

But if we view the question as Tennent ('10) has done, and especially as Loeb, King and Moore ('10) have done, the solution seems to be much more adequate. Certain characters are dominant, others are recessive. In the experiments which we are discussing, the dominant characters are the presence of posterior epaulettes and of posterior pedicellaria. They are always transmitted to the descendants, either through the father or through the mother; the green pigment is a recessive character. Its appearance is always prevented, either by the maternal, or by the paternal influence.



To this assertion I must add two remarks :

(1) The dominant character is not necessarily transmitted in its entirety ; it may be lessened by a contrary influence. An example of this is to be found here in the fact that the posterior epaulettes are less perfectly developed in hybrids than in pure larvæ. This statement does not contradict Mendel's law of dominance. It is necessary to remark that one is not working on adult individuals with fully developed characters, but on larvæ. Now Lang ('08) has observed in his crossings of *Helix* that in young hybrids the rate of development of certain characters is less than in pure individuals. Moore ('12) remarks and explains the same fact in Echinoderm hybrids.

(2) It would seem necessary to admit that the dominant characters are variable, that is to say, that if they are normally transmitted to hybrids, this may not be the case if certain factors vary. The present experiments perhaps give us an example of this. The posterior pedicellaria is, in fact not always present in the hybrids. Too much importance must not, however, be attached to this example, because one cannot absolutely determine what relationship exists between the posterior pedicellaria of *Echinus esculentus* or *acutus* and the posterior spine of *E. miliaris* ; and we do not know enough of the changes which occur in these elements in the pure larvæ. Better examples of the variability of the dominant characters are to be found in the researches on echinoderm hybrids, which have been already published. According to Tennent ('10) the heredity of the characters varies with the alkalinity of the water. According to Vernon ('00), Steinbrück ('02), Doncaster ('04), and Herbst ('06, '07), this heredity varies with the seasons or more probably with the temperature. It seems, therefore, that one may take it that a normally dominant character is weakened under certain conditions and that a recessive character may, under certain conditions, be strengthened and be transmitted as a dominant character.

We should like to compare our results and conclusions with

those obtained by other experimentalists, but in most of the experiments the question of dominant characters was not considered, and the examination of their works would go beyond the limits of this paper. I will simply say that my conclusions agree with those of Loeb, King and Moore ('10), of Moore ('12), and of Tennent ('10). They would also probably agree with those of Vernon ('00), Doncaster (04), and Herbst ('06, '07).

It only remains for me to speak of the difference which exists between the results of my experiments and of the earlier experiments of Shearer, De Morgan, and Fuchs ('11). This year these authors obtained results which are partly opposed to the results they had obtained in former years, and which, as far as I can judge from their preliminary notes ('Nature,' June 27th, 1912) are fairly analogous to the results which I am now describing. They believe that this difference is due to the fact that certain external circumstances affect the sexual cells of *Echinus miliaris*.

It seems difficult to admit this hypothesis because, contrary to their observations, I have noticed that the eggs of *E. miliaris*  $\times$  *miliaris* which developed were relatively more numerous than the eggs of *E. acutus*  $\times$  *acutus*; and that the larvæ of *E. miliaris*  $\times$  *miliaris* were very easy to rear; in the same way the larvæ of *miliaris*  $\text{♀}$   $\times$  *acutus*  $\text{♂}$  were relatively more numerous than those of *acutus*  $\text{♀}$   $\times$  *miliaris*  $\text{♂}$ . If one studies the question with regard to the dominant characters, one comes to a conclusion which is quite opposed to the one given by these authors.

In their account of this year's experiments one notices that these authors deal with two dominant characters (posterior epaulettes and posterior pedicellaria) belonging to *E. acutus* and one recessive character (green pigment) belonging to *E. miliaris*. In the experiments of former years these dominant characters were not transmitted by the sperm of *E. acutus*, nor was the recessive character crushed by the action of the sperm of *E. acutus*. It appears, therefore,

that some influence weakened the force of the sexual cell of *E. acutus* or strengthened the force of the sexual cells of *E. miliaris*.

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## EXPLANATION OF PLATE 16,

Illustrating the paper by Dr. G. Debaisieux on "The Experimental Hybridisation of *Echinus miliaris*, *Echinus esculentus*, and *Echinus acutus*."

### LIST OF ABBREVIATIONS EMPLOYED.

*ant. ep.* Anterior ciliated epaulette. *ech.* "*Echinus rudiment*."  
*gr. pig.* Green pigment. *l. ped.* Lateral pedicellaria. *post. ep.* Posterior ciliated epaulette. *p. ped.* Posterior pedicellaria. *sp.* Adult spine.

[N.B.—Only the green pigment is indicated in the drawings; the red pigment, which is present in all the larvæ figured, is left out.]

Figs. 1-2.—Pluteus of *Echinus miliaris* × *E. miliaris* viewed from the ventral surface. *Gr. pig.* The green pigment.

Fig. 3.—Pluteus of *E. esculentus* × *E. esculentus* viewed from the right side. After the drawings of Mr. MacBride.

Fig. 4.—Hybrid pluteus of *E. miliaris* ♀ × *E. esculentus* ♂, from

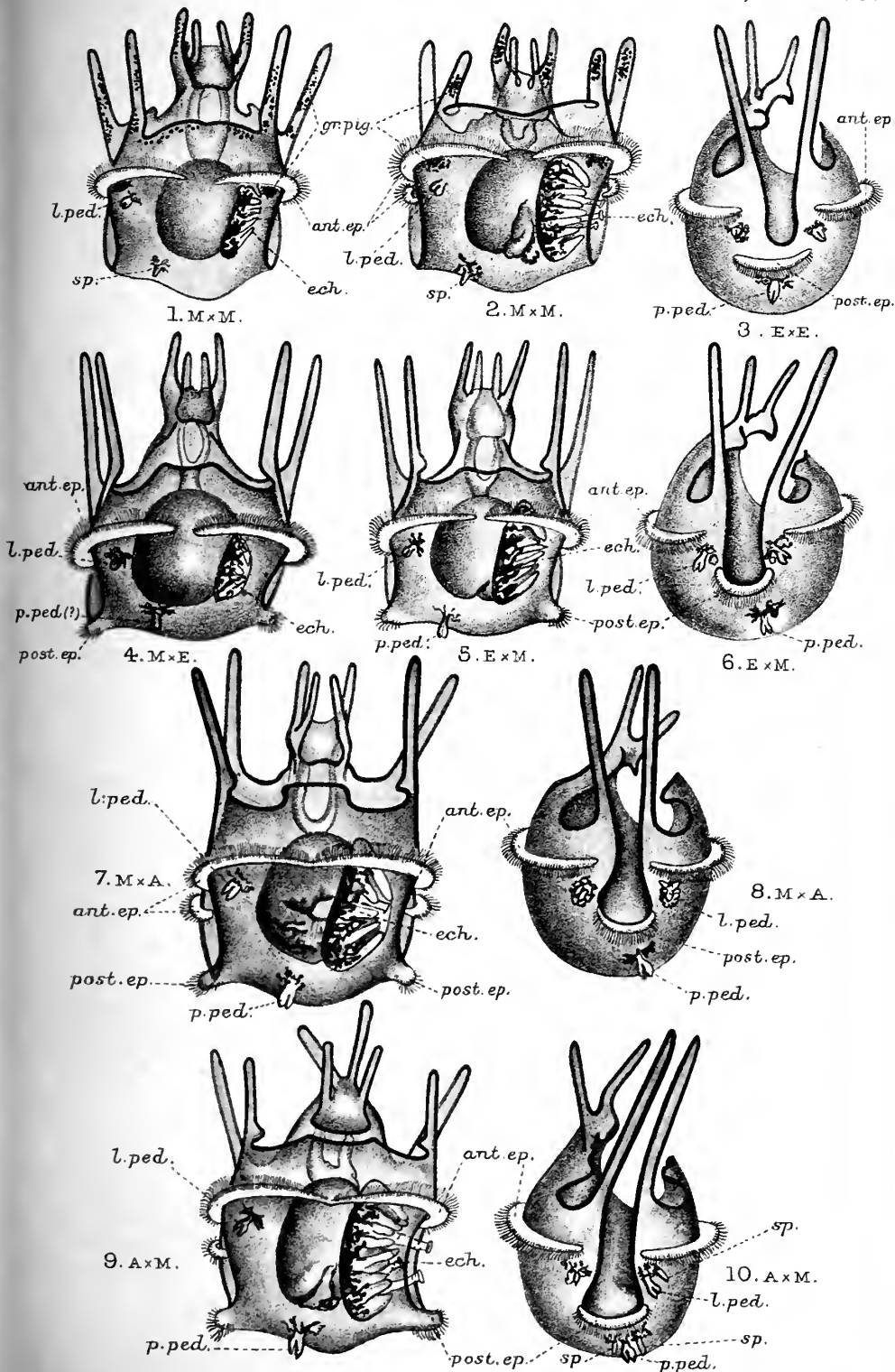
the ventral surface. *p. ped.*<sup>1</sup> Knob-like outgrowth probable rudiment of posterior pedicellaria.

Figs. 5-6.—Hybrid plutei of *E. esculentus* ♀ × *E. miliaris* ♂, viewed, the first from the ventral, the second from the right side.

Figs. 7-8.—Hybrid plutei of *E. miliaris* ♀ × *E. acutus* ♂, viewed, the first from the ventral, the second from the right side.

Figs. 9-10.—Hybrid plutei of *E. acutus* ♀ × *E. miliaris* ♂, viewed, the first from the ventral, the second from the right side.









## On Paternal Characters in Echinoid Hybrids.

By

Cresswell Shearer, Walter De Morgan, and H. M. Fuchs

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With Plates 17 and 18 and 4 Text-figures.

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(From the Laboratory of the Marine Biological Association,  
Plymouth).

### I. INTRODUCTION.

LAST autumn we published a paper (12) giving a brief summary up to date of some three years' work on the hybridisation of Echinoderms. The experiments were made with the three species of *Echinus* found at Plymouth. From a study of the late larval characters of the pure forms and of the hybrids we arrived at the conclusion that the inheritance of these characters was invariably maternal—that is to say, that the late larval hybrid always resembles its mother. Since the date of the publication of our preliminary paper we have continued our experiments, and have had occasion to repeat all our crosses. To our surprise, however, the behaviour of some of the hybrids has differed greatly this season from that of previous years. In late larval life some of the hybrid crosses have shown as strictly a paternal inheritance as in previous years they showed a maternal one. It may therefore be of interest to other investigators in this field of work if we publish a short account of these new facts before the completion of our full paper.

In our preliminary paper we have clearly pointed out that the very conflicting results which have been obtained by different investigators in Echinoderm hybridisation are in large part due to the study of insufficiently definite characters.

These characters are those developed in the early pluteus—in particular the larval skeleton. This is a structure which varies very readily with the state of health of the organism, and the variations in one form frequently tend to give it a shape resembling that of another. For instance, the skeleton of the antero-lateral arms of the *Echinus pluteus* is a simple rod, but in abnormal cases a lattice form is developed like that of *Sphærechinus*. For this reason it is evident that the skeleton is an unsatisfactory index of heredity, and it would seem to be doubtful if much of the previous work on inheritance in the very early pluteus is of substantial value.

In the forms which we have studied we have been forced to adopt larval characters later than those hitherto used as criteria of inheritance. This has been rendered possible by the elaboration of methods of rearing healthy larvæ in quantity up to and beyond metamorphosis. In the advanced plutei of *Echinus* we have found characters which do not vary in the parental forms and which are present in the one species and absent in the other.

Our experiments have been made with the three common English species of *Echinus*, namely *E. esculentus*, *E. acutus* and *E. miliaris*. The characters, of which we have investigated the inheritance, have been as follows: *E. esculentus* and *E. acutus* both develop posterior as well as anterior ciliated epaulettes (Pl. 17, figs. 2, 3). *E. miliaris*, on the other hand, has no posterior epaulettes, but two pairs of green pigment-masses are formed in the anterior epaulettes (Pl. 17, fig. 1). All the hybrid plutei have in previous years been purely maternal with respect to these characters. Thus in the crosses *E. esculentus* ♀ × *E. miliaris* ♂ (Pl. 17, fig. 5) and *E. acutus* ♀ × *E. miliaris* ♂ (Pl. 17, fig. 4) the posterior ciliated epaulettes are developed, although not to so great an extent as in *E. esculentus* or *E. acutus*, but the green pigment-masses are absent. In the crosses *E. miliaris* ♀ × *E. esculentus* ♂ (Pl. 18, fig. 8), and *E. miliaris* ♀ × *E. acutus* ♂ (Pl. 17, fig. 6), on the other hand, the posterior epaulettes are absent and the green pigment is present.

During 1909-1911 no exceptions were found to this rule of maternal inheritance, and in the present year the crosses *E. esculentus* ♀ × *E. miliaris* ♂ and *E. acutus* ♀ × *E. miliaris* ♂ have behaved as before. It is, however, in the hybrids with *E. miliaris* ♀ that we have obtained different results. All the cultures of *E. miliaris* ♀ × *E. acutus* ♂ have shown a paternal inheritance. Thus, in Pl. 18, fig. 7, the green pigment of *E. miliaris* is absent, while the posterior epaulettes of *E. acutus* are present. Again, in the cross *E. miliaris* ♀ × *E. esculentus* ♂, with an exception, all the fertilisations gave paternal larvæ (Pl. 18, fig. 9). It seems, then, that it is the *E. miliaris* eggs which are this year in general unable to transmit the characters of the species to the hybrids.

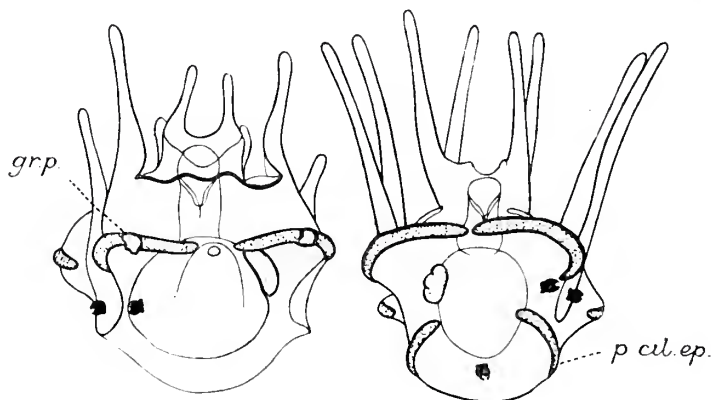
In former years cultures of *E. miliaris* have always shown a more rapid rate of growth in the laboratory than the other species. This, we have suggested in our preliminary paper, is possibly due to the fact that, *E. miliaris* being a shore form, laboratory conditions may be more suited to it than to *E. esculentus* or *E. acutus*, the habitat of which is in deeper water. Not only did *E. miliaris* develop rapidly, but any crosses into which it entered had their rates of growth accelerated. This year, however, this species can only be obtained in a late stage with great difficulty, and its rate of growth is slower than that of *E. esculentus*, *E. acutus*, or any of the hybrids.<sup>1</sup> With regard to the latter, when *E. miliaris* is used as the male parent, fertilisations can easily be made, and the inheritance is, as usual, maternal. When, however, *E. miliaris* eggs are used in the cross, only a very low percentage are fertilised, and the inheritance is, as stated above, paternal. A few exceptions only have been found to this. In one experiment only a high percentage of the *E.*

<sup>1</sup> Since the above was written this matter has been brought up at the Dundee meeting of the British Association. Prof. MacBride stated there that he had this year bred *E. miliaris*, obtained from Plymouth, at the Imperial College of Science in London, and that the larvæ could be raised as easily as in former years.

*miliaris* eggs fertilised with *E. esculentus* sperm, and some of the larvæ were paternal, others maternal.

From the above considerations it would seem that some condition in the environment has affected the *E. miliaris*, so that the eggs are unable to develop healthily when fertilised with sperm of the same species, and are usually incapable of transmitting their characters to hybrid offspring.

TEXT-FIG. I.



*E. miliaris.*

*E. esculentus.*

Diagram to show the characters of the late plutei of *E. miliaris* and *E. esculentus*. *gr. p.* Green pigment-mass. *p. cil. ep.* Posterior epaulette.

## II. SUMMARY OF EXPERIMENTS.

In our preliminary paper we have sufficiently indicated the methods which have been adopted in carrying out this investigation. It only remains to add here that rigid precautions have always been taken against any possible error in the fertilisations. As in all our previous work proper controls have been kept. In addition, our results of this year are not based alone upon the examination of a few culture jars, but on a large number of experiments extending over several months.

The following is a brief description of the late larvæ of the three pure forms and of the hybrid crosses.

TEXT-FIG. 2.

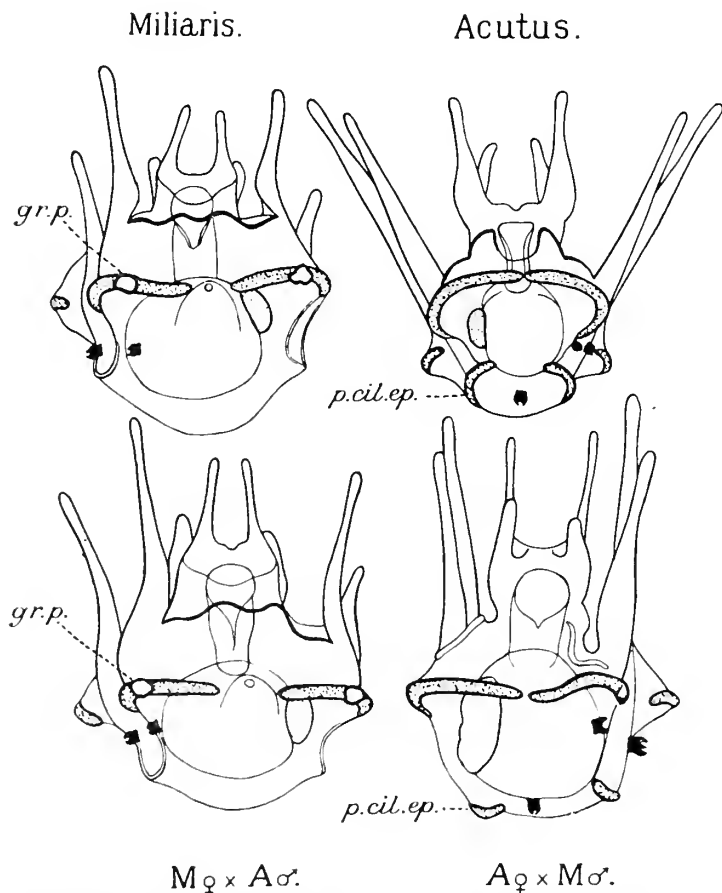


Diagram to show the inheritance of the late larval characters in hybrids between *E. miliaris* and *E. acutus* during 1909-11.  
*gr p.* Green pigment-mass. *p.cil.ep.* Posterior ciliated epaulette.  
 The two upper figures represent the unhybridised larvæ.

The pluteus of *E. esculentus* reaches the 8-armed stage at about a fortnight after fertilisation. The age of a pluteus is, however, seldom a good indication of its stage of development, as the rate of growth in different cultures

varies within wide limits. The larva (Pl. 17, fig. 2) has a body which is deeper than wide, the posterior pole being flattened. As soon as the eight arms are developed the ciliated band around the anterior edge of the larva thickens, four crescentic bands being abstricted. These are the anterior epaulettes, and by means of their strong cilia they form the principal

TEXT-FIG. 3.

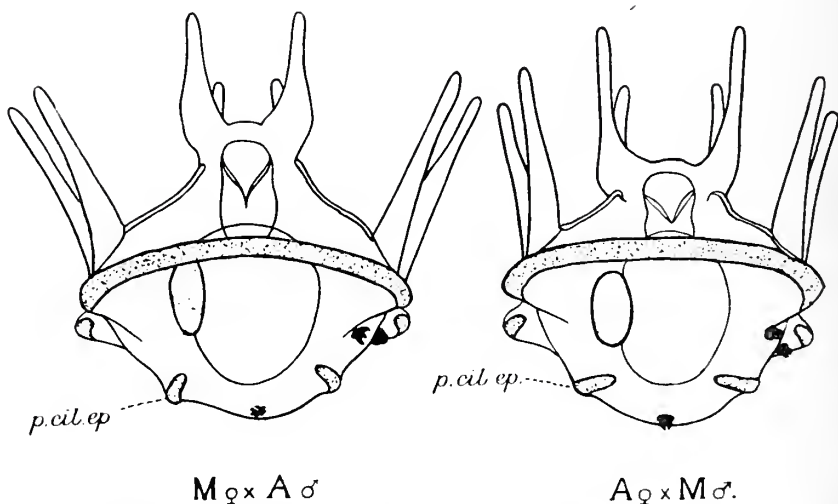


Diagram to show the inheritance of the late larval characters in hybrids between *E. miliaris* and *E. acutus* during 1912 (cf. Text-fig. 2) and absence of the green pigment-masses in the hybrids when *E. miliaris* was maternal. *p. cil. ep.* Posterior ciliated epaulette.

locomotory organ of the fully formed pluteus. Eventually they coalesce to form one continuous band. At the end of about three weeks a pair of posterior ciliated epaulettes is formed. The presence of these structures in this species is one of the characters of which we have investigated the inheritance (Text-fig. 1). A pair of pedicellariæ appear dorsally and ventrally on the right side of the larva, opposite to the Echinusrudiment, which is now well advanced. A posterior pedicellaria is usually present as well. It is also developed in *E.*



TEXT-FIG. 4.

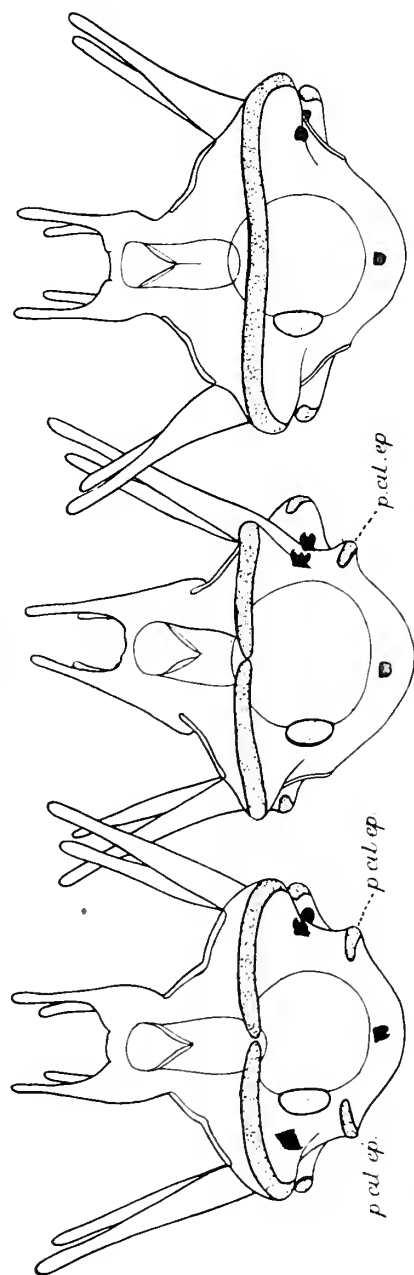


Diagram to show an exceptional case of the inheritance of the late larval characters in the cross *E. esculentus* ♀ and *E. miliaris* ♂, in 1912. *p. cul. ep.* Posterior ciliated epaulette.

*acutus*, but is never present in *E. miliaris*. The inheritance of this pedicellaria follows that of the epaulettes, but it is not a very reliable feature, as it occasionally fails to develop in pure *E. esculentus*, *E. acutus*, or the hybrids. The larva is studded with reddish-brown pigment spots, which are concentrated at the tips of the arms and along the ciliated epaulettes.

The pluteus of *E. acutus* (Pl. 17, fig. 3, and right upper figure in Text-fig. 2) closely resembles that of *E. esculentus*, and develops posterior epaulettes. It has, however, a rather smaller body, with more slender and divergent arms. Owing to the similarity in essential features between this species and *E. esculentus*, hybrids between them give no information of hereditary value.

The *E. miliaris* larva (Pl. 17, fig. 1, and Text-fig. 1) is of a different general shape from the two described above, the body being wider and having a domed posterior end. The arms are comparatively short. A dorsal and a ventral pair of green pigment-masses are developed in the anterior epaulettes. This pigment is completely absent in the plutei of *E. esculentus* and *E. acutus*. No posterior epaulettes are developed in *E. miliaris*. The presence of the green pigment and the absence of the posterior epaulettes are the important features of this larva for the study of heredity (Text-fig. 1).

This year (Text-fig. 3), as in previous seasons (Text-fig. 2), hybrids of the cross *E. acutus* ♀ × *E. miliaris* ♂ (Pl. 17, fig. 4) have developed maternal characters. The posterior epaulettes are present, and there is no trace of the green pigment-masses.

Plutei of the cross *E. esculentus* ♀ × *E. miliaris* ♂ (Pl. 17, fig. 5) have similarly always been purely maternal with respect to the same characters. In one experiment, however, all the larvæ had the usual material absence of green pigment, but some developed both posterior epaulettes, some neither and others had one on one side of the body only (Text-fig. 4).

With the cross *E. miliaris* ♀ × *E. acutus* ♂ we have, however, obtained totally different results this season from

those of former years. In 1909-1911 the hybrid larvæ were always maternal (Pl. 17, fig. 6, and Text-fig. 2), having no posterior ciliated epaulettes, but developing the green pigment. In all the experiments of this year only a very low percentage of the eggs would fertilise with the foreign sperm. Perfectly healthy larvæ were obtained from these fertilisations, but in all the cultures they showed an inheritance which was the exact reverse of the previous years. Posterior epaulettes were developed, but no green pigment, showing that the larvæ inherited their characters through the sperm (Text-fig. 3).

From the cross *E. miliaris* ♀ × *E. esculentus* ♂ we have also obtained results which conflict with those of other years. Formerly the characters of the hybrid plutei were always maternal (Pl. 18, fig. 8). Posterior epaulettes were absent, and green pigment was present. This year we have had as great a difficulty to fertilise the *E. miliaris* eggs with *E. esculentus* sperm as with *E. acutus* sperm. In one case only did we get a good fertilisation, about 80 per cent. of the eggs segmenting. In cultures made from this cross some of the hybrid larvæ were maternal, others paternal. In all other experiments the percentage of eggs which fertilised was twenty or less. Nevertheless, these

	1909, 1910 and 1911.		1912.	
	Posterior epaulettes.	Green pigment-masses.	Posterior epaulettes.	Green pigment-masses.
<i>E. esculentus</i> , ♂ } <i>E. esculentus</i> , ♀ }	+	0	+	0
<i>E. miliaris</i> , ♂ } <i>E. esculentus</i> , ♀ }	+	0	+	0
<i>E. miliaris</i> , ♂ } <i>E. miliaris</i> , ♀ }	0	+	0	+
<i>E. esculentus</i> , ♂ } <i>E. miliaris</i> , ♀ }		+	+	0

larvæ developed quite healthily, but their characters were paternal (Pl. 18, fig. 9). The posterior ciliated epaulettes were developed, but the green pigment was totally absent.

On p. 345 is given in tabular form a summary of the inheritance of the late larval characters investigated. The difference between the inheritance this year and that in previous years can be seen at a glance. The crosses between *E. miliaris* and *E. acutus* gave the same results as those between *E. miliaris* and *E. esculentus*.

### III. DISCUSSION.

In a comparison of the general results of hybridisation among Echinoderms with that in other divisions of the animal and vegetable kingdoms it is striking that the products of reciprocal crosses are, in the former case, so frequently unlike one another. In the species of *Echinus* with which we have experimented, the characters investigated have, until this year, always been inherited through the maternal parent. Such behaviour seems to be of quite a different order from that of pairs of characters which follow the usual Mendelian law, in which cases the hybrids of one cross resemble those of its reciprocal, irrespective of the sex of the parent forms. Prof. Punnett has pointed out to us a case among plant hybrids which would seem to be parallel with this parental inheritance in Echinoderms. In crossing two species of *Oenothera*, de Vries (15) found that the hybrids were always strongly paternal. Hybrids of the cross *O. biennis* ♀ × *O. muricata* ♂ resembled *O. muricata* while those of the reciprocal resembled *O. biennis*; so that in this case the hybrids showed an inheritance through the male germ-cells. It is interesting to note here that he kept each of the hybrid strains for four generations without observing any alteration in their characters.

If data could be obtained of inheritance through more than one generation in Echinoderms, it might be found that the facts could be interpreted on Mendelian lines. As, however,

up to the present no hybrids have been raised to sexual maturity, any suggested Mendelian explanation must remain unconfirmed. Nevertheless we would suggest that the following hypothesis might be put forward to express the purely maternal inheritance. Suppose that the presence of a pair of posterior ciliated epaulettes (P) and the absence of the same (p) are a pair of allelomorphic characters. Then the germ-cells of *E. acutus* will all have P and those of *E. miliaris* will all have p. Suppose, moreover, that in hybrids when P comes in through the male it is recessive to p, but when through the female it is dominant,

then <i>E. acutus</i> ♀ ×	{	recessive p through the ♂	}	the hybrid having posterior epaulettes;
<i>E. miliaris</i> ♂ receives		dominant P through the ♀		
and <i>E. miliaris</i> ♀ ×	{	recessive P through the ♂	}	the hybrid having no posterior epaulettes.
<i>E. acutus</i> ♂ receives		dominant p through the ♀		

If this hypothesis were true and segregation took place, the  $F_2$  generations from both reciprocal crosses would have to be composed of larvæ in equal numbers with and without the posterior epaulettes. If, on the other hand, there were no segregation, all the  $F_2$  individuals would be alike and similar to the  $F_1$  hybrid from which they were bred.

We have explained above that two of our crosses have this year shown a complete reversal in the inheritance of the parental characters. While in hybrids between *E. esculentus* or *E. acutus* and *E. miliaris*, in which the latter was used as the male parent, the characters were maternal as formerly, when *E. miliaris* was used as the female the hybrids this season usually resembled the father. Formerly they had been invariably maternal. This complete change in heredity from one year to another seems to be without parallel in hybrids other than those among Echinoderms. Using the same notation as above we should have to suppose that this year P behaves as a simple dominant over p.

As we have emphasised above, previous investigators in Echinoderm hybridisation have used characters which were not definite enough to give unquestionable results. Never-

theless the frequent inconsistencies between the conclusions of different experimenters may in part be of the same nature as our change in inheritance. In 1889 Boveri (2), working at Naples, found that hybrids of the cross *Sphærechinus* ♀ × *Echinus* ♂ were all intermediate in their characters. Seeliger (11) made the same cross at Trieste in 1894, but found that in every culture some of the larvæ were paternal. Morgan (10) repeated the work at Naples in 1895 and substantiated Seeliger's conclusions. It is possible that the inheritance of parental characters in this cross does not remain the same during a series of years. Similarly the opposite results obtained with hybrids between *Strongylocentrotus franciscanus* and *S. purpuratus* by Hagedroon (7) and Loeb, King and Moore (9), who worked at Pacific Grove, Cal., in two consecutive years, may possibly both be correct. In any case our results serve to emphasise the fact that it is necessary to repeat the same experiments many times and to extend them over a considerable period. If the investigation had been made at Plymouth during this year alone, the conclusion would probably have been arrived at that the characters of *E. esculentus* and *E. acutus* are dominant over those of *E. miliaris*. We know, however, that this has not been the case in preceding years.

Vernon (14), working at Naples in 1900, found that the inheritance in hybrids between *Strongylocentrotus* and *Sphærechinus* was different according to the time of year at which the crosses were made. In spring the hybrid larvæ resembled *Strongylocentrotus*, while in summer they were like *Sphærechinus*. *Strongylocentrotus* was found to be much riper in the spring than in the summer, and accordingly Vernon concluded that the dominance of the one species over the other was controlled by the relative ripeness of the sex cells used to make the cross. Doncaster (3), however, found that the cause of the change in inheritance was the difference in temperature at the two seasons. We cannot compare our results strictly with those described above. The breeding period of the three species of

*Echinus* found at Plymouth is relatively short so that the crosses can be made at one season of the year only. It appears to be plain, however, that the relative ripeness of the germ-cells used in our experiments is of no account. For, during the four seasons of this investigation, hybrids made at the commencement or end of the breeding season have not differed from those made at the period of maximum maturity.

Since it was in crosses in which *E. miliaris* was used as the female parent that the inheritance was different this year from that in previous seasons, there would appear to be some alteration in the eggs of this species. This is borne out, not only by the fact that the normally fertilised eggs of *E. miliaris* develop slowly and irregularly, but also that in the only hybrid cross in which the percentage of fertilisation was high, some of the larvæ showed the usual maternal inheritance. Since this species has failed to develop healthily it might seem that the eggs were immature and that the breeding period had for some reason been postponed. But even if this were so, previous experience has shown us that immature eggs, although they may give unhealthy larvæ, do not alter the inheritance in hybrids. On the whole it seems most probable that some factor in the environment has affected the metabolism of *E. miliaris* in its habitat this year, so that the condition of the female germ-cells is changed.

In 1906 Kupelwieser (8) fertilised *Echinus* eggs with *Mytilus* sperm and obtained hybrid larvæ which were purely maternal. He found, however, that the chromatin of the sperm had not fused with that of the eggs, so that there had been no true fertilisation. In the earlier stages of our investigation this suggested to us that the invariable maternal dominance which we found might be a case of virtual parthenogenesis similar to that of Kupelwieser. Godlewski (6) fertilised *Echinus* eggs with *Antedon* sperm but obtained a result which differed from that of Kupelwieser. The larvæ were maternal, but he showed that there had been a true fusion of the nuclei, the male chromatin taking an active part in the segmentation mitoses. This result showed



that the sperm chromatin has not necessarily any influence on the structure of the hybrid. Other investigators in Echinoderm hybridisation who have found a purely maternal inheritance (Driesch (5) and Hagedoorn (7)) have not investigated the cytology of their crosses. In order to decide whether or not there was a true fertilisation in our experiments we handed over hybrid eggs fixed in the early segmentation stages to Doncaster and Gray. A preliminary account of the results of their investigation was published last autumn (4). At that date they had only examined one cross into which *E. miliaris* entered, namely *E. acutus* ♀ × *E. miliaris* ♂. In these hybrids there was a true fusion of the male and female pronuclei, so that this seemed to be a case parallel with that of Godlewski.

Baltzer (1), working at Naples in 1908, found that in his crosses, although there was a true fusion of the pronuclei, a varying number of chromosomes were thrown out in the early segmentation divisions. He also claimed that chromatin was eliminated as late as the blastula stage. Tennant (13) has also found elimination of chromosomes in the early stages. It is possible that such a rejection of chromatin may take place at an early or a late stage in our *Echinus* crosses, and that sometimes paternal chromatin may be thrown out while at others maternal. A change in elimination of this nature might be correlated with the change in inheritance which we have described above.

A full account of the investigation of Doncaster and Gray, which was made on identical material from which some of our crosses were raised, will appear shortly in this journal.

During this summer we have investigated the inheritance of characters in the young hybrid sea-urchins after metamorphosis. An account of this work will be given in a later paper. A method has been found of feeding and raising the urchins in the laboratory and a number of these have now reach a considerable size. It is hoped that we may obtain from them a second generation.

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EXPLANATION OF PLATES 17 AND 18,

Illustrating the paper by Messrs. Cresswell Shearer, Walter De Morgan and H. M. Fuchs on "Paternal Characters in Echinoid Hybrids."

LETTERING.

*a. cil. ep.* Anterior ciliated epaulette. *ech. r.* Echinus-rudiment.  
*gr. p.* Green pigment. *p. cil. ep.* Posterior ciliated epaulette. *ped.* Pedicellaria.

PLATE 17.

Fig. 1.—*Pluteus* of *Echinus miliaris*. Dorsal view. Note green pigment-masses in anterior epaulettes and absence of posterior epaulettes.

Fig. 2.—*E. esculentus*. Dorsal view. Green pigment absent. Posterior epaulettes present.

Fig. 3.—*E. acutus*. Dorsal view. Green pigment absent. Posterior epaulettes present.

Fig. 4.—*E. acutus* ♀ × *E. miliaris* ♂. Ventral view. Maternal characters.

Fig. 5.—*E. esculentus* ♀ × *E. miliaris* ♂. Dorsal view. Maternal characters.

Fig. 6.—*E. miliaris* ♀ × *E. acutus* ♂. Dorsal view. Maternal characters.

PLATE 18.

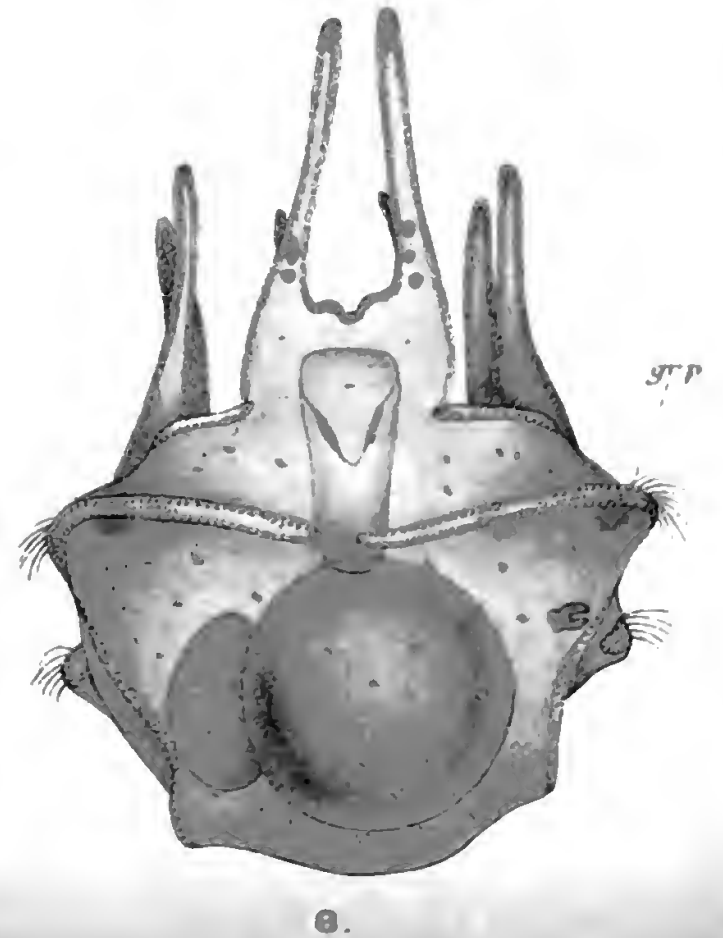
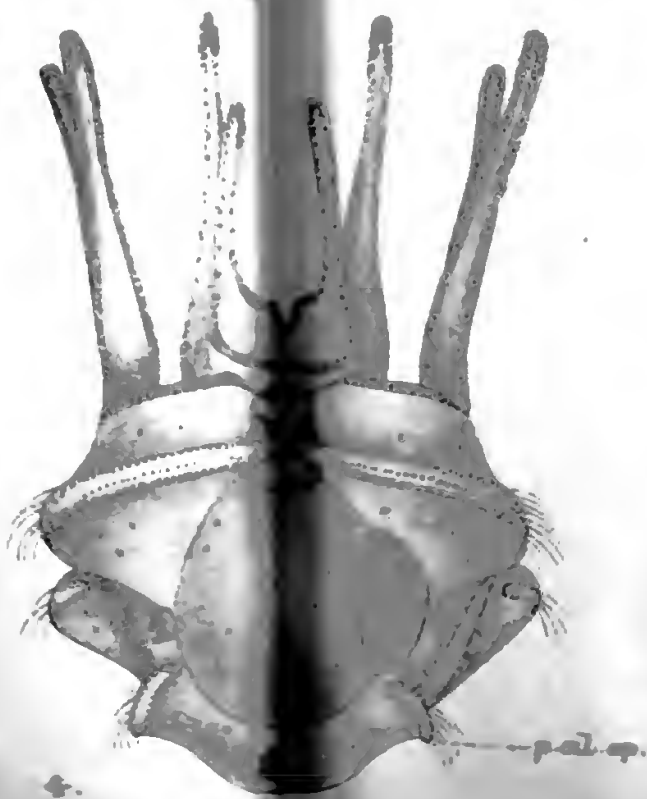
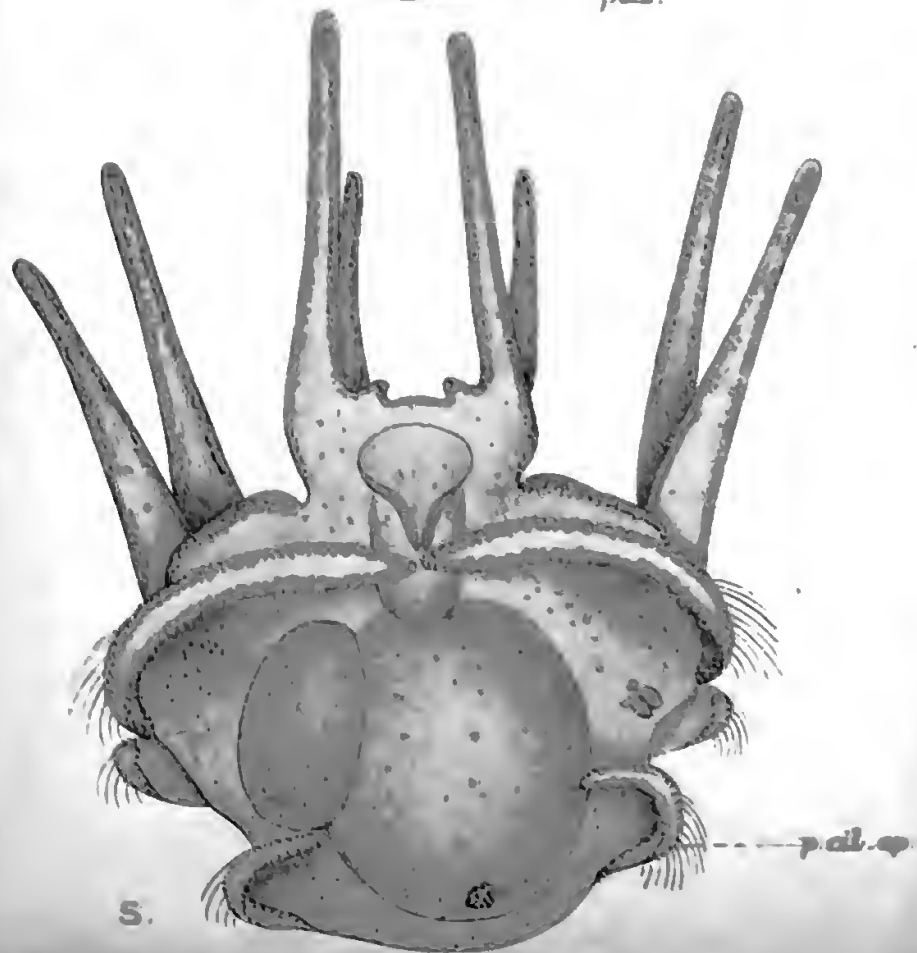
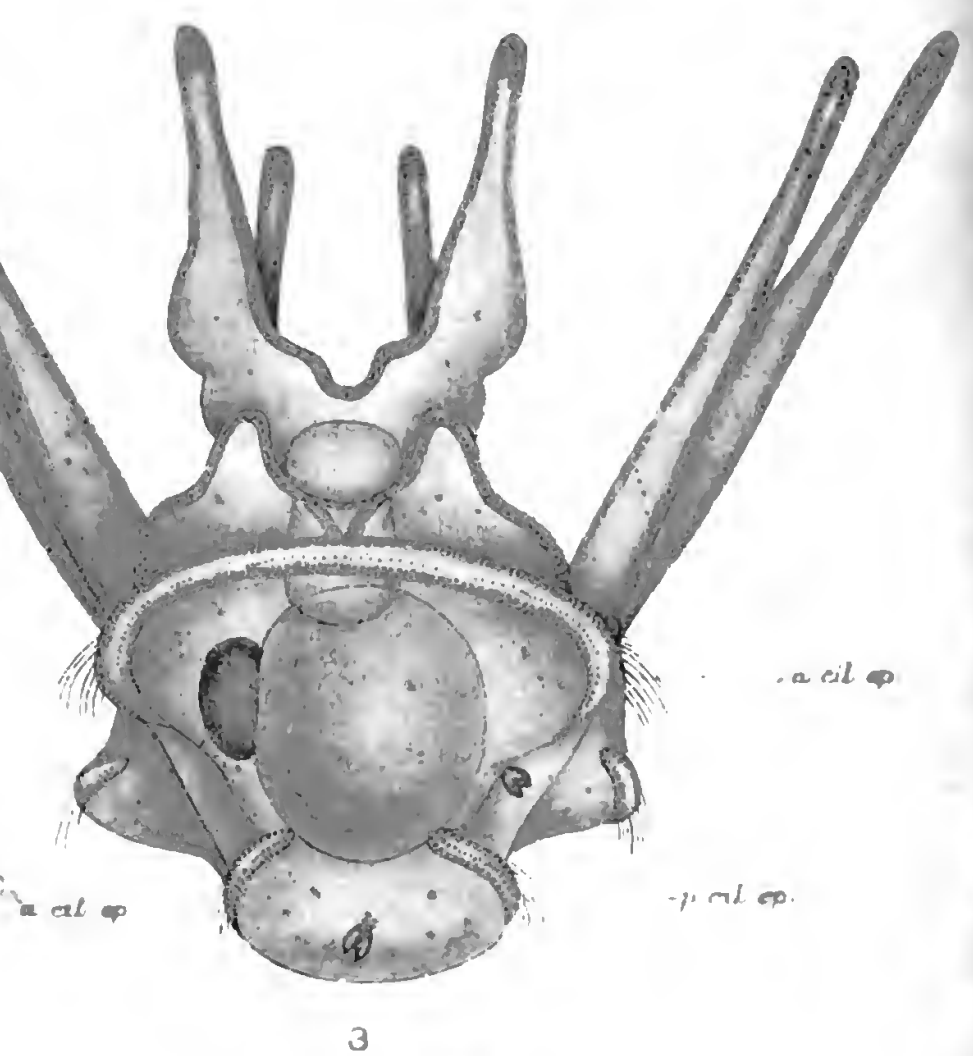
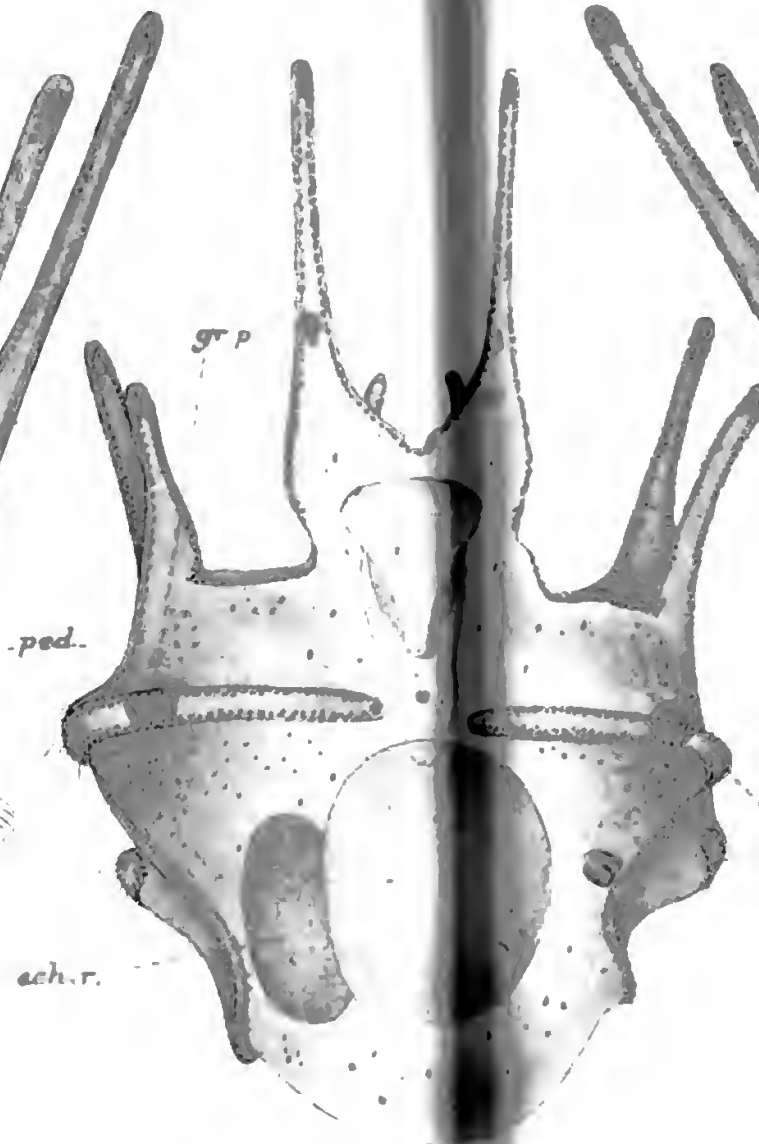
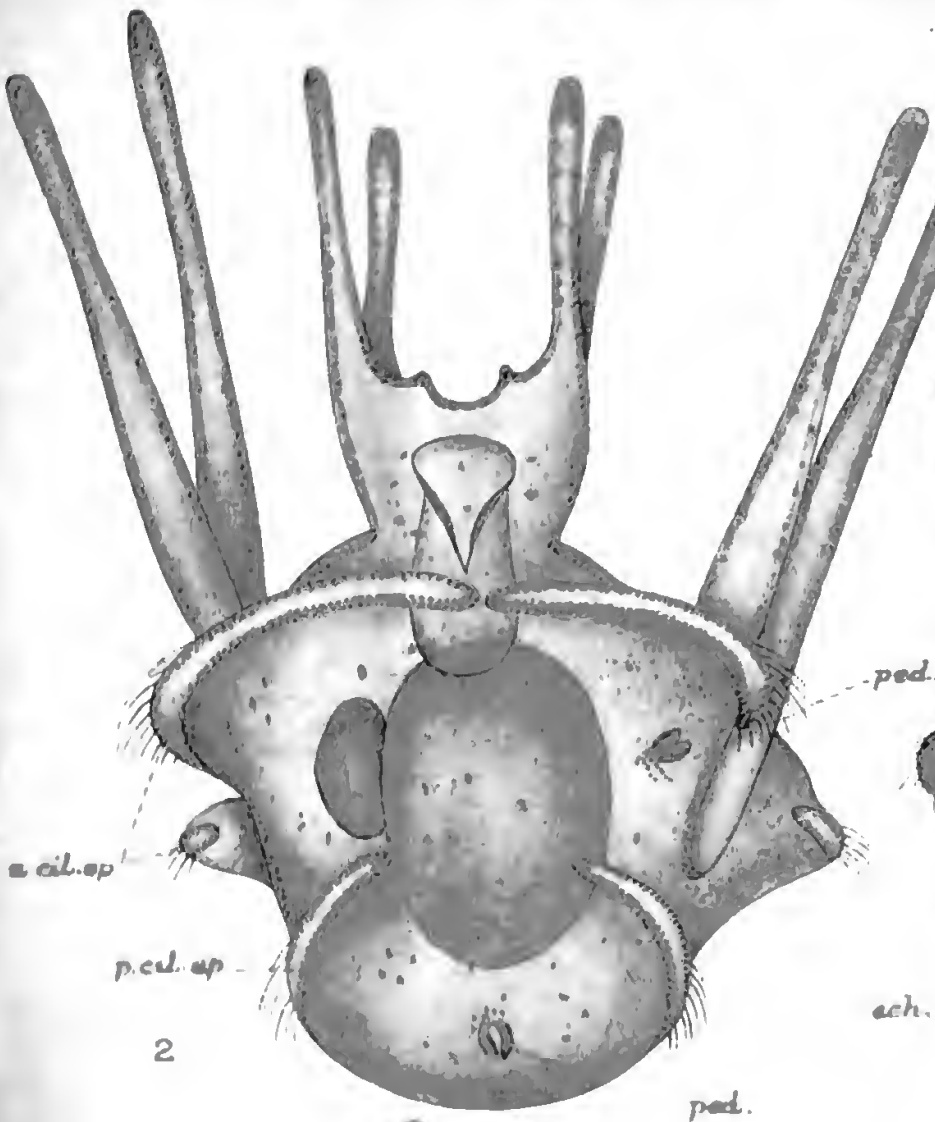
Fig. 7.—*E. miliaris* ♀ × *E. acutus* ♂. Ventral view. Paternal characters

Fig. 8.—*E. miliaris* ♀ × *E. esculentus* ♂. Dorsal view. Maternal characters.

Fig. 9.—*E. miliaris* ♀ × *E. esculentus* ♂. Dorsal view. Paternal characters.



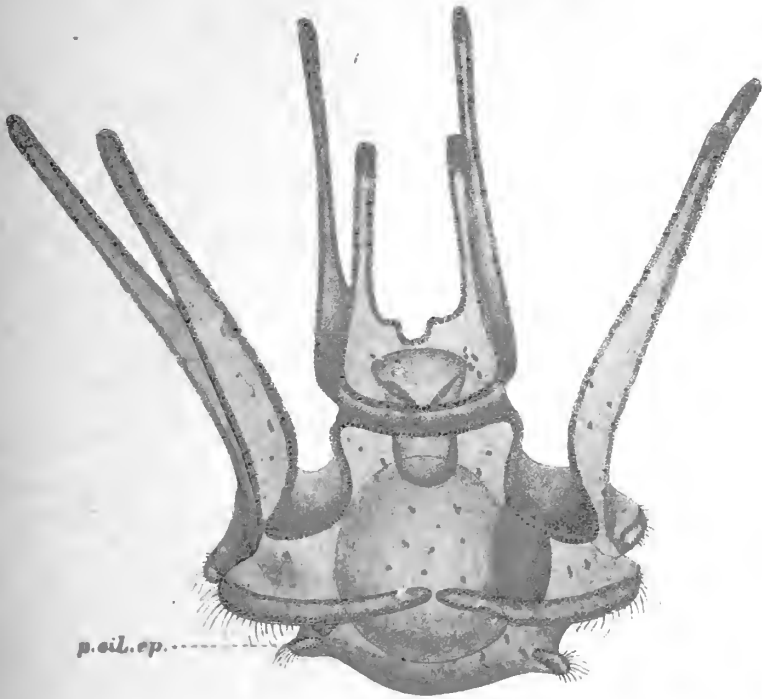








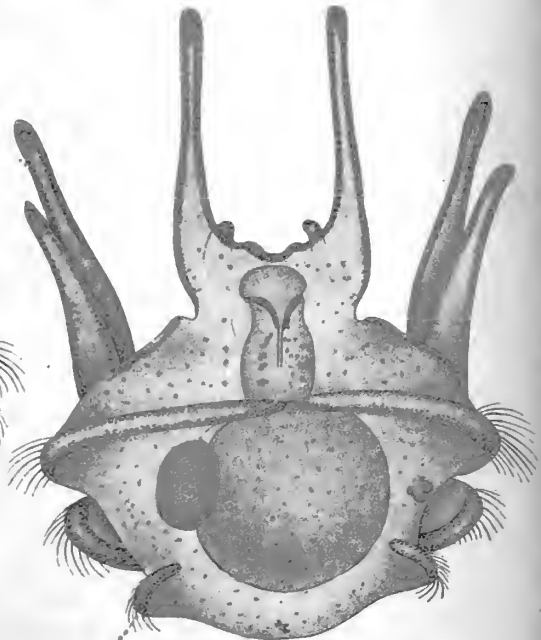




7.



8



9.



## On the Morphology of the Leishmania of Italian Kala-Azar.

Third Communication<sup>1</sup>: Cytological Researches on Leishmania in Cultures.

By

**Dr. Arrigo Visentini**

(From the Zoological Department of the Lister Institute of Preventive Medicine and the Institute of Comparative Anatomy of the University of Rome).

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With Plates 19 and 20.

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RESEARCHES on the minute structure of Protozoa, although initiated in comparatively recent times, have already contributed to science a considerable number of facts of great interest, especially from the point of view of general cytology. But while observations conducted with the most delicate methods of cytological technique are feasible up to a certain point when dealing with Protozoa of a certain size, they meet with no slight difficulties when dealing with micro-organisms which, like the species of *Leishmania*, are of the smallest dimensions. When, more than a year ago, I made successful cultures of *Leishmania* on the medium of Novy, McNeal and Nicolle, by means of splenic juice obtained by puncture of the spleen of a young kala-azar patient of Bovalino Calabro, my teacher, Prof. Grassi, repeatedly recommended me to undertake the present investigations with the special object of studying the little-known structure of the blepharoplast and the rhizoplast, with

<sup>1</sup> The author's two previous communications on this subject were published in the 'Archiv f. Schiffs- und Tropenhygiene,' xiv (1910), Beiheft 4, and 'Pathologica,' iii (1912).

regard to which Grassi, in collaboration with Foa, has described a whole complicated system of organellæ in *Jœnia*, *Trichonympha*, and other Protozoan parasites of Termites.

My investigations, begun in the Laboratory of Comparative Anatomy at Rome, were continued by me during my stay in the Institut für Schiffs- und Tropenhygiene at Hamburg, and were resumed, after some interval, in the Protozoological Department of the Lister Institute of London by the express desire and advice of Prof. Minchin. To Profs. Grassi and Minchin, who have directed and guided me in a field of study almost new to me, I desire to express my gratitude.

Investigations on the finer structure of *Leishmania* in the *Leptomonas* form are almost entirely new, especially for the *Leishmania* which is the specific agent of kala-azar in the countries of the Mediterranean basin and which goes under the name of *L. infantum*. None of those who have occupied themselves with the morphology of this Protozoon in the flagellate stage have made use of those methods of histological technique which alone permit a study of the minute structure of Protozoa, and have limited themselves rather to describing the various forms which are met with in the culture-tubes, some of them trying especially to reconstruct the developmental cycle of the parasite. But the data with regard to the developmental cycle of the parasite are more than ever uncertain and contradictory; as is known for other flagellate Protozoa, the cultural forms are for the most part aberrant or resting forms, and it is probable that many of those described in the cultures of *Leishmania* may be regarded rather as degenerative forms. On the other hand, concerning the structure of the parasite there have resulted many inexact observations.

Of great interest are, above all, the questions regarding the significance of the blepharoplast and its exact relations with the flagellum. Rogers has described in the first days of the development of *L. donovani* in acidulated citrated blood, a body which stains with eosin, for the most part rounded or oval, closely apposed to the blepharoplast, which is now

attached to the periphery or approximated to it. This "eosin-body" (named by Leishmann "flagellar body") is found in the elongated forms at the anterior extremity of the parasite, always maintaining the same relations with the blepharoplast. It could be better described as an area coloured in pink around the blepharoplast and the rhizoplast, and it is supposed by Franchini to have been found ultimately expelled from the parasite in the dried smears fixed in alcohol and stained with Giemsa's stain.

Wenyon also has occupied himself with the structure of *L. tropica* in cultures, and my researches, more complete in many points, will permit me, as will be seen in the sequel, to follow out still further the intimate morphological relations which exist between these two species, so closely allied, the one the specific agent of oriental sore, the other of kala-azar.

I have had at my disposition three sources of cultures. Besides that obtained by myself and mentioned above, I have received other cultures from the Pasteur Institute of Paris, which originated from Nicolle in Tunis, and others very recently from the Clinica Pediatrica of Palermo. To Messrs. Mesnil and Jemma, who so kindly sent me them, I return my best thanks.

The results obtained from these different cultures agree perfectly, as was to have been expected, so that in the descriptions of my observations I refer to the one or the other indifferently.

When preparations are to be made of flagellates in a culture on blood-agar, it is a most important precept that the smears should be very thin, otherwise the flagellates for the most part do not become differentiated, and Protozoa also stain in an incomplete manner; moreover the examination is hindered by the presence of hæmoglobin particularly, which takes an intense colour, and in which the Protozoa are imbedded.

The smears on a cover-glass or slide were made with a small drop of the culture by means of another cover-glass or slide, smearing the material in a backward direction.

The cultures were always very rich in parasites and of very recent date (from two or three days to a week), in order to avoid the presence of dead or altered forms and to obtain the greatest possible number of dividing forms.

It is an essential precept to fix the preparation immediately, before the smear dries, because, as in the case of very many other Protozoa, the *Leishmanias* become deformed rapidly, assuming always a form more stumpy or even rounded; the method of drying, besides itself altering the form and the structure, is not a sufficiently rapid means of fixation. It is for this reason that those authors who have made use only of dried smears, stained with Giemsa's stain, have given incomplete and inexact descriptions, as recently Franchini. In particular, with this method the rhizoplast is not always successfully demonstrated, and never the exact connection of the latter with the kinetonucleus.

For fixation I have made use largely of Schaudinn's alcoholic sublimate and of Maier's fluid, which is a slight modification of the former; of osmic acid, followed by subsequent fixation in methyl alcohol, absolute alcohol, or Schaudinn's fluid; and of Flemming's fluid and Hermann's fluid, always used cold. After fixation in mixtures containing sublimate, I have followed usually the technique indicated by Giemsa for wet smears and sections, that is to say, treatment with iodine solution and afterwards with hyposulphite; but I abandoned this procedure when, like Rosenbusch, using a solution of sublimate in which there were no crystals, I made the fixation last but a short time.

Two methods of staining have given me especially good results—Giemsa's method for wet smears and Heidenhain's classic iron-haematoxylin; I have not had equally good differentiation of the stain with the abbreviated procedure of Rosenbusch, which, however, gives good results and has the advantage of saving much time. The differentiation of the preparations is especially delicate and difficult, requiring to be checked by frequent examination under the microscope; for this purpose I have made use with advantage of the water-

immersion objective of Zeiss (2.5 mm.), with which it is possible to control step by step the most minute structural details.

The cultures of *Leishmania* develop rapidly, and flourish in the Novy-McNeal-Nicolle medium prepared according to the formula of Nicolle, which, I think, it worth while to record briefly here:

Agar-agar, not washed	. . . . .	14	gram.
Sodium chloride	. . . . .	6	„
Distilled water	. . . . .	900	c.c.

The mixture is dissolved and sterilised in the autoclave, taking care to add afterwards some sterile water, as much as has been lost during the sterilisation. The agar, not alkalinised nor neutralised, is placed in the sterile tubes in the desired quantity (I make use of test-tubes 12 cm. in length and about 2 cm. in diameter, and place in each 4 c.c. of agar), and then sterilised afresh for ten minutes. The tubes can be kept a long time, provided that all desiccation of the agar is avoided by closing them with a rubber cap and keeping them in the ice-chest, sheltered from light.

At the moment of preparing the substratum the agar is re-dissolved by boiling water and allowed to cool to 45°–55° C., at which temperature is added to it, with proper precautions to ensure sterility, one third of its volume of defibrinated rabbit's blood, introducing it with a sterile pipette holding 10 c.c., which I change two or three times during the operation. The agar and the blood are mixed by shaking the test-tube well, but care should be taken that excessive froth is not formed. The tubes are then placed on an inclined plane in order that the blood-agar may solidify in a form like the mouthpiece of a flute, and left thus about twelve hours. Closed again with rubber caps they are placed in the incubator at 37° C., in order that the water of condensation may collect in good quantity. There is no advantage in leaving them in the thermostat two or three days, as Nicolle prescribes, since the water of condensation always diminishes a little.

Tubes prepared in this manner can be kept in the cold chamber at a temperature of about 0° C. for some months ready



for use, but care must be taken to maintain them for half-an-hour at 21° prior to planting them. Nicolle, Massaglia and others have employed aseptic puncture of the heart in order to obtain the rabbit's blood in a sterile manner. Although this method, after some experience, succeeds fairly well, it never allows so large a quantity of blood to be obtained as from the carotid, and I have adopted with constant success this procedure, which, moreover, is used also in Mesnil's service at the Pasteur Institute in Paris. The anæsthetised rabbit is fixed in a manner which exposes the throat; the skin, previously disinfected and carefully shaved, is cut with aseptic precautions along the median line and the trachea is laid bare, taking care not to wound the blood-vessels; at the side of the trachea the carotid is easily found and is isolated from the nerves without injuring them, and is tied with sterile silk as near as possible to the skull. It is seen that at the level of the thyroid gland the thyroid artery arises from the carotid, and this is isolated and tied near the carotid and cut above the knot in order to have it as a firm stalk for holding and raising the carotid with a forceps later on, without diminishing its lumen by taking hold of its wall directly. As a greater precaution at this point I pour a little alcohol on the artery, and then I cut it near the cranial ligature and collect the jet of blood in a sterile Erlenmayer flask containing the usual glass beads in order to defibrinate the blood. With the forceps which holds the carotid by the stalk formed by the stump of the thyroid artery, the carotid can be held within the orifice of the Erlenmayer flask without touching its walls (which, moreover, are previously sterilised in the flame) until the blood is drawn.

By this method as much as 55-60 c.c. of blood can be obtained from a rabbit of medium size without the animal dying, if care be taken to tie the cut carotid quickly and to sew up the wound rapidly without chloroforming the animal too much. The operation, when a certain expertness has been acquired, may last only a few minutes.

It is necessary that I should make some remarks with

regard to nomenclature, since the various structural elements of *Leishmania* have received various denominations, especially from the Italian authors; moreover, it is necessary to put aside names which have different meanings and may produce confusion, and to restore the nomenclature of *Leishmania* to that which is in scientific use for the cell-body of flagellate Protozoa allied to the genus *Leishmania*.

Since my observations, like those of Wenyon for *Leishmania tropica*, enable me to affirm the nuclear nature of the blepharoplast-nucleus of the *Leishmania* of Italian and Tunisian kala-azar in the flagellate form of the *Leptomonas* in cultures, one should abandon, as has been done for the other Protozoa, the names "nucleolus," "micronucleus," "centrosome" and "extra-nuclear centrosome," which record a past uncertainty of knowledge with regard to the blepharoplastic nucleus and engender a grave confusion with other constituent parts of the cell endowed with quite other functions. There remain in common use the denominations kinetonucleus and blepharoplastic nucleus or blepharoplast simply; but Minchin and Woodcock and other English authors indicate very properly by the name blepharoplast the basal granule (Rosenbusch) from which the flagellum takes origin, and which is situated usually in the zone of the nuclear sap of the kinetonucleus.

The principal nucleus or trophonucleus (Woodcock) of *Leishmania* is of vesicular type, and, especially in the preparations fixed wet and stained with hæmatoxylin by Heidenhain's method (figs. 19-46), presents the typical structure of the nucleus of trypanosomes and other allied Protozoa. It is constituted by a membrane, well defined and of a certain thickness, which encloses a clear space, the so-called zone of nuclear sap, and at the centre a granule of chromatin stained a deep black and of compact structure, the karyosome. In the preparations differentiated for a long time in iron-alum, it is possible to distinguish in some *Leishmanias* a very small granule, the centriole, in the interior of the karyosome.

From the karyosome very delicate filaments (linin) can sometimes be seen to arise, connecting the karyosome to the nuclear membrane in a radiate manner, and showing here and there very minute granules connected to one another in their turn by transverse threads. On the inner surface of the nuclear membrane, granules and dots (Chromatinkomplex of Schaudinn) are observed united to the karyosome by delicate filaments. But in general the linin-framework and the chromatin of the nuclear sap-zone are very feebly developed in the principal nucleus of *Leishmania* and the granules extremely minute.

With the method of Giemsa, after wet fixation (fig. 3) it is also possible to demonstrate the minute structure of the nucleus in all its constituent parts; on the other hand, in dried smears Giemsa's stain usually colours the sap-zone also, so that the nucleus appears as a rounded homogeneous body, in the interior of which some granules of chromatin more intensely stained can be made out with difficulty.

In the smears stained with iron-haematoxylin there are sometimes found in the nuclear sap-zone of the principal nucleus one or even two granules intensely stained, larger than those which are met with normally along the threads of the linin-network and easily distinguished from them. Their number, one or two, is especially characteristic, and in fig. 42 I have represented a nucleus in which the two granules are connected by a very delicate filament, not to be confounded, it appears to me, with a filament of linin. We are dealing here, perhaps, with the centriole or extra-karyosomic centrosome dividing into two for the first time as a prelude to the division of the whole nucleus; or is this a phenomenon of chromatinic reduction, like others of which I shall give an account in the sequel?

The kinetonucleus, situated between the trophonucleus and the anterior extremity of the body, but nearer to the former than to the latter, appears composed mainly of a fusiform or oval body situated with its greater diameter in a transverse direction, compact and intensely stained, representing the

karyosome of the kinetonucleus. This body presents itself sometimes as two rounded granules quite distinct and united by a zone stained more feebly, instead of as a single dense oval body; but in that case there exist already two rhizoplasts or even two flagella, and I believe that this is always a case of the beginning of the process of division, which commences, as is known, in the kinetonucleus.

Around the karyosome a clear circular zone is observed, corresponding to the nuclear sap-zone of the trophonucleus and delimited by a membrane, very delicate but always quite evident. In this zone it is sometimes possible to distinguish also some fine chromatinic granules without linin-threads. At the periphery of the clear zone there is found anteriorly as a rule a granule more or less evident, the basal granule or blepharoplast properly so-called, from which the rhizoplast arises.

The flagellum of *Leishmania* never ends at the anterior pole of the parasite, as has been described by observers whose methods of staining have been defective, but is always continued into the body of the Protozoon in a posterior direction and along the axis of the parasite as far as the kinetonucleus. There is thus a rhizoplast constantly present in the *Leishmania*, a structure which, moreover, exists already in the forms of *Leishmania* in the human body, as I have described and figured in one of my preceding publications.

To establish exactly the precise relations and connection of the flagellum with the kinetonucleus is not always easy.

In the greater number of cases the flagellum ends in the blepharoplast proper or basal granule, which, as I have stated, is found anteriorly in the sap-zone of the kinetonucleus, more or less near to the karyosome, sometimes adhering to it. When there is a certain amount of space between the two it is possible to demonstrate a structure in the form of a finely striated cone, as if it were constituted by very minute fibrils, which unite the karyosome to the blepharoplast itself. This is just as is observed sometimes in many trypanosomes and in *L. tropica*.

In other cases the flagellum is united directly to the kinetonucleus, and in many cases (figs. 5, 10-13, 26, 31) the impression is obtained that it arises directly from it. Then there is no basal granule in its usual position. The possibility cannot, of course, be excluded absolutely that in these cases the basal granule and point of origin of the flagellum are found in a slightly lower plane, behind the karyosome which lies over it, but the figures are very suggestive of the interpretation that the flagellum is really in continuity with the karyosome, within which in such cases the blepharoplast would also be found. Moreover, to the latter is attributed usually the significance of the centriole of the motor nucleus; and the assumption that the blepharoplast is found in some cases in the interior of the karyosome explains also, as we shall see in the sequel, the behaviour of these various elements in the process of division.

It is known that in *Leishmania* the process of division begins usually by that of the kinetonucleus; nevertheless there are cases in which a precocious multiplication of the principal nucleus takes place, so that there may be in the same parasite two trophonuclei completely distinct and the first stages of division of the motor nucleus. In the kinetonucleus the process begins by the division of the blepharoplast, followed immediately by that of the flagellum; in this way are produced forms in which, as in figs. 7, 8, 16, 18, there are two rhizoplasts which unite anteriorly, and which are continued by a single flagellum; in these cases either there are already two basal granules in the sap-zone, or the doubled rhizoplast is continued into the interior of the karyosome, and this is almost the rule.

The splitting of the flagellum proceeds rapidly, and from it results almost always a normal flagellum, and, near it and parallel to it, one much shorter (figs. 6, 9, 10, 11, 13, 14, 31, 33, 34, 35), as is observed frequently also in trypanosomes. In most cases, however, after the new rhizoplast has been formed, it detaches itself at its distal extremity from the other, and, when separated, gives origin to the new flagellum,

which begins to protrude from the anterior pole of the parasite and subsequently grows continually in length (figs. 4, 5, 9, 10). At the same time or immediately afterwards the division of the karyosome takes place.

It should be noted at this point that I have never found true and proper figures of karyokinesis of the kinetonucleus with the formation of an achromatinic spindle.

All these stages of division are, of course, only to be observed in preparations fixed by wet methods and suitably stained; dried preparations give only a very inexact and incomplete idea of the whole process (figs. 6 and 7).

The successive stages of division of the kinetonucleus can be followed step by step in figs. 9-18 and 31-35. The karyosome becomes elongated and constricted in the middle, and the two halves which are thus produced travel further and further apart, remaining connected by a very slender filament, which persists during the division of the trophonucleus; in this way a true centrodemesmosis of the kinetonucleus is formed. At this stage there exist already two rhizoplasts and two flagella, which usually become detached from the two halves, still connected, of the karyosome.

Special interest attaches to the *Leishmania* represented in fig. 34; in this specimen the two flagella take origin from two granules situated outside the two halves of the karyosome and respectively very close to each of them; a very delicate achromatinic filament unites the two granules or blepharoplasts properly so-called, from which, as I have said, the flagella originate. This would prove that the blepharoplasts have the significance of centrioles of the motor nucleus, and that actually in an early period of the division they are found in the interior of the karyosome, at the point when the flagellum of the flagella already formed seem to take origin from the interior of the karyosome itself; subsequently, however, the blepharoplasts regain their normal position in the sap-zone near the membrane.

The division of the principal nucleus follows a process perfectly analogous, and takes place in all its stages within the

nuclear membrane. I believe that the appearances shown in figs. 28, 29 and 30 should be interpreted as early forms of multiplication. In these the nuclear chromatin is not collected, as in the majority of cases, in a compact mass forming a single karyosome at the centre, but is broken up into granules and short rods which perhaps correspond to chromosomes, and which, distributed at first irregularly in the sap-zone, afterwards arrange themselves so as to constitute a single elongated mass of irregular contour, disposed transversely to the longer axis of the organism and always contained within the nuclear membrane. Subsequently the chromatin collects at the extremities in two masses, which remain united by a thread that becomes continually more attenuated. Here also there is a true and proper centrodesmose with an achromatinic filament which stains strongly and resists prolonged differentiation with iron-alum; it unites, as is seen specially in fig. 35, two stained granules, which correspond, I believe, to the centrioles of the daughter-nuclei.

The nuclear membrane persists throughout and can be seen well in the preparations stained with iron-haematoxylin, and better still in those stained by Giemsa's method. In the latter it is seen that the membrane, like the whole nucleus, assumes at first an oval form, then is gradually constricted in half in the form of a figure of eight, disposed transversely to the greater axis of the *Leishmania*; when the two halves are joined by the delicate filament, it constitutes the membranes of the two rounded daughter-nuclei (figs. 11, 12, 13, 14, 15, 16, 17, 18, 32, 33, 34, 35). Between the latter the centro-desmotic filament breaks and the division is complete. Meanwhile the division of the protoplasm has already begun (fig. 17), or follows rapidly.

In spite of many long and patient observations I have never come across forms of division, either of the tropho-nucleus or the kintonucleus, in which the presence was observed of an achromatinic spindle and of a typical equatorial plate, such as has been described in trypanosomes



by Rosenbusch and some other authors, and I believe that I can exclude completely this type of the process of division in the case of *Leishmania* in the flagellated *Leptomonas* form. In this case there is a process of mitosis without formation of an achromatic spindle giving rise to two daughter-cells exactly equal. I wished, however, to reproduce in the interesting figures 44, 45 and 46 some nuclei which present exactly the phenomena described recently by Horta and Machado (15) in *Trypanosoma chagasi* n. sp., a trypanosome of fishes in Brazil, and interpreted, I believe, erroneously as a complete process of heteropolar division. In support of my judgment there is also the fact that the authors cited have never seen two distinct nuclei arise from the supposed division, or subsequent fission of the protoplasmic body of the parasite; just as I have never found this to occur in the *Leishmania*.

I believe rather that we are dealing simply with a reduction of nuclear chromatin, with no direct relation to the process of multiplication. Without wishing to suggest phenomena of maturation in relation with a hypothetical sexual differentiation, I am persuaded that these facts of nuclear reduction (since it is a question without doubt of a reduction of chromatin) are to be placed rather in the group of phenomena which Richard Hertwig terms "Kernplasma-Relation" (14).

Having studied the process of division in many preparations, it has never happened to me to come across forms which would suggest that the kinetonucleus performs the function of a centrosome in the division of the principal nucleus, a function ascribed to it by Moore and Breinl, on which ground they have named the kinetonucleus the "extra-nuclear centrosome." The nuclear structure and the mode of division of the motor nucleus are sufficient to exclude such an interpretation, but it should also be recognised, from the examination of the preparations, that the division of the trophonucleus and that of the kinetonucleus appear up to a certain point to be independent the one of the other, so much

so that the one may be already finished when the other is beginning, and if they take place synchronously, they may be found in different stages. Moreover, I have never observed a fusion of the kinetonucleus with the principal nucleus, as Row has described in the cultures of *Leishmania tropica*, not even in the first days of the development, when the flagellated forms are arising from those of the spleen.

With regard to the first origin of the flagellum my observations are very scanty, since I have only twice obtained cultures of spleen-material, but I am able to confirm the descriptions given by other authors, and to state precisely the manner in which the flagellum exists.

Beginning with the small forms of *Leishmania* from the internal organs of kala-azar patients, and especially those in which the rhizoplast can already be demonstrated (figs. 7, A, B, of my memoir [26]), the next stage has a more rounded form, or is already pear-shaped (see fig. 38) with a more distinct rhizoplast. The flagellum begins to protrude from the body of the parasite and becomes continually longer, while the organism becomes pyriform and elongated, and the kinetonucleus, which at first was found by the side of the nucleus, passes anteriorly to it. In this way there are flagellates of more or less elongated form with one end pointed and the other, the anterior end, rounded, and also long and very slender forms such as that represented in fig. 21.

The protoplasm of *Leishmania* in cultures appears, with the best fixatives, very finely granular, and contains frequently here and there granules of various sizes, identical with the volutin-grains of trypanosomes. After prolonged coloration with Heidenhain's hæmatoxylin of smears fixed in Schaudinn's fluid, the protoplasm often assumes a variegated appearance, with patches intensely coloured and separated by clear spaces.

I have never observed in the protoplasm the existence of a filament uniting the kinetonucleus to the trophonucleus, as described by Prowazek and some of his pupils, amongst them also recently Chagas in *Trypanosoma cruzi*. After

fixation, especially with liquids containing osmic acid (osmic acid and Schaudinn's fluid, Flemming's and Hermann's fluids), I have observed the presence in the space between the trophonucleus and kintonucleus and very close to the latter of a corpuscle with contours not very sharply defined, for the most part rounded and without an internal structure, and stained more feebly than the nuclear karyosome (see figs. 36, 37, 21). In preparations fixed with osmic acid and Schaudinn's fluid I have demonstrated fairly frequently a filament which, starting from the posterior pole of the parasite, traverses the body in a spiral and ends near the motor nucleus, in some cases in the body just mentioned, which in my opinion can be identified with that described by Novy (19) and by Sangiorgi (24) in trypanosomes.

I have been led to these investigations above all by the very important works of Grassi and of Foa on the structure of the Protozoa parasitic in Termites, studies which have opened an horizon of research with regard to the motor apparatus of flagellate Protozoa. I do not know whether the various parts described in the Leishmanias as rhizoplast and kintonucleus can be considered analogous to the organellæ described by Grassi and Foa, and named by Janicki the "parabasal apparatus." It may be that the ill-defined body situated near the kintonucleus can be compared to the parabasal body. But after the examination of many preparations with more or less effective methods of staining, I am certainly inclined at present to consider the spiral filaments demonstrated by me after osmic fixation rather as folds of the ectoplasm, artificial products of the preparation, than as true and proper structural elements.

I have not omitted to undertake repeatedly examinations in the fresh state in physiological salt solution, and also in the same liquid containing a very weak solution of picric acid, so dilute as to permit the flagellates to continue living for a certain time. The Leishmanias then show exactly the same form as in the stained preparations when the fixation has not been preceded by desiccation ; there is perhaps a still greater

uniformity in the morphology of the parasite, with prevalence of the typical spindle form with the anterior extremity rounded, while the stumpy and rounded forms are rarer, those that occur being for the most part in process of division. In the interior of the body the trophonucleus and the kinetonucleus can be distinguished clearly, especially in forms which do not contain many granules.

It is a noteworthy fact that the two nuclei of the *Leishmania* present an identical appearance, as a dark point surrounded by a clear halo. When the motility of the Protozoa is diminished and the examination of the structural details is rendered possible, it is often possible to make out that the flagellum is continued as a rhizoplast into the body of the organism as far as the kinetonucleus, ending at the boundary of the clear halo of the latter. In the space between the trophonucleus and the kinetonucleus it is not infrequently possible to observe a body slightly darker than the protoplasm with contours not well defined, without a halo and without perceptible structure, exactly similar to the body which is made evident by the iron-haematoxylin stain, especially after osmic fixations. My attention, as is natural, has been drawn particularly to the search for something which would correspond to the axostyle ("mestolo," Axenstab), that is to say, the so-called axial rod in the protoplasm between the nucleus and the posterior extremity, but I have never succeeded in observing it. Perhaps the rhizoplast is homologous with it?

Observation in the fresh state and with very powerful magnification is of course very tiring, and it is above all very difficult to pick out details of structure in organisms so minute, but it is to be hoped that further researches will lead to new and definitive knowledge with regard to the finer structure of these flagellate Protozoa.

THE LISTER INSTITUTE.

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## EXPLANATION OF PLATES 19 AND 20,

Illustrating Dr. Arrigo Visentini's paper "On the Morphology of the Leishmania of Italian Kala-azar."

## PLATE 19.

Figs. 1-18.—Flagellated forms of *Leishmania infantum* from cultures stained with Giemsa's stain.

Figs. 4-18 show various stages in the process of division.

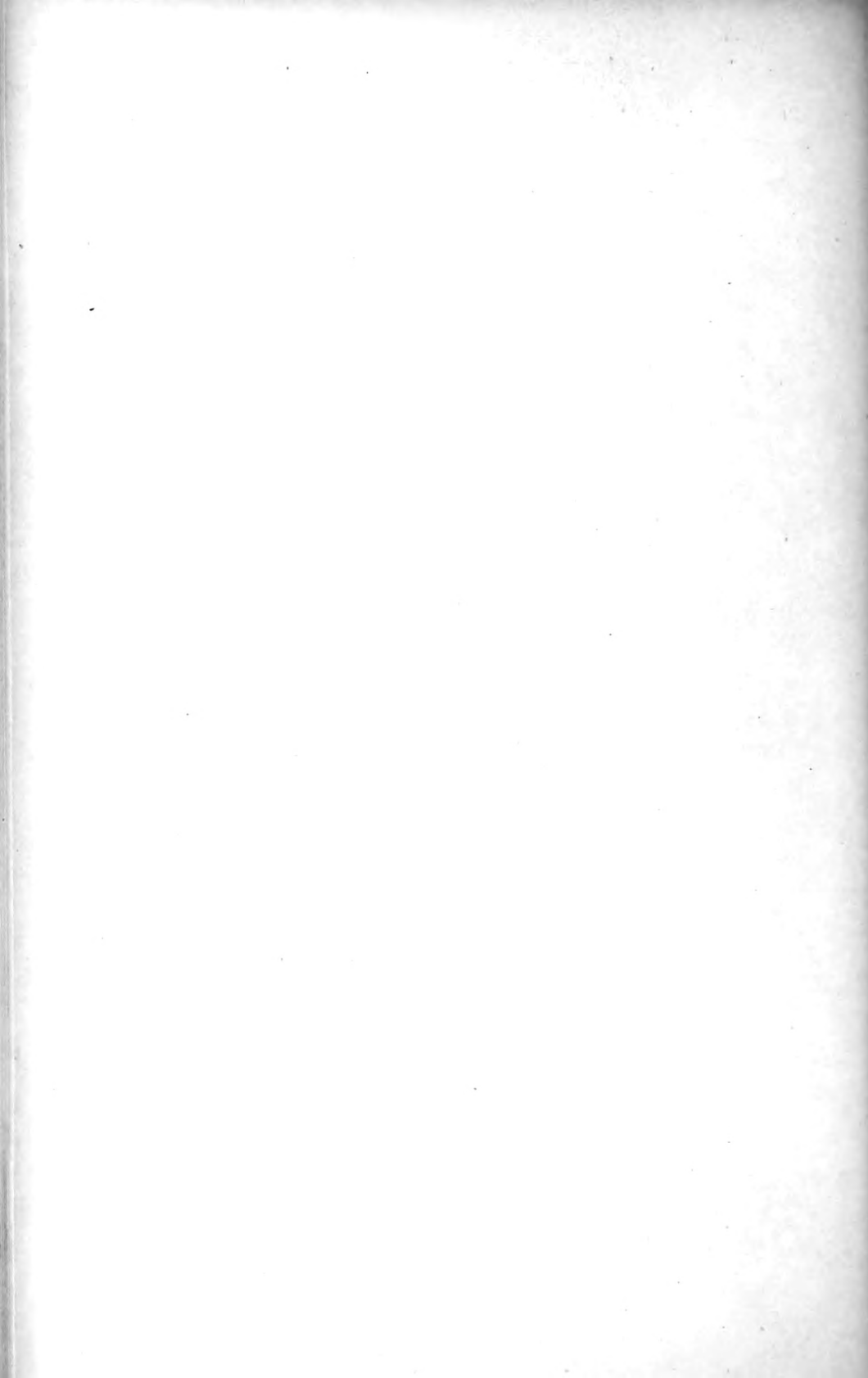
All figures drawn with the camera lucida at a magnification of 2000 diameters.

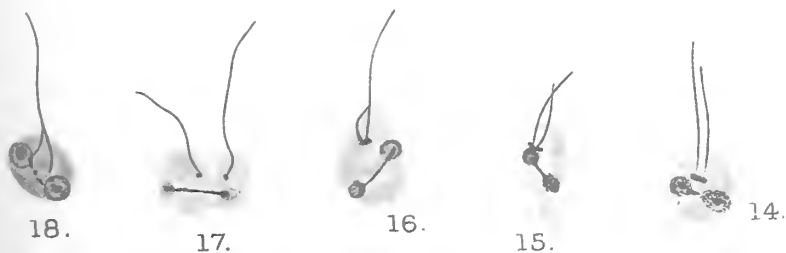
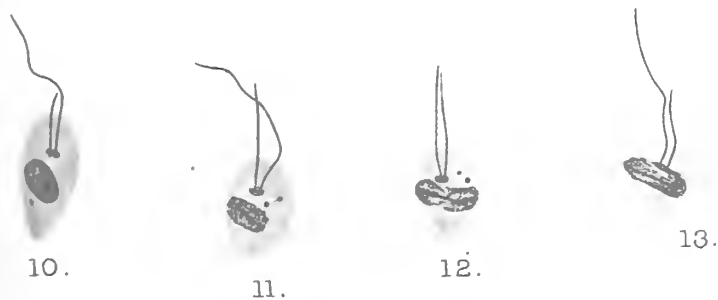
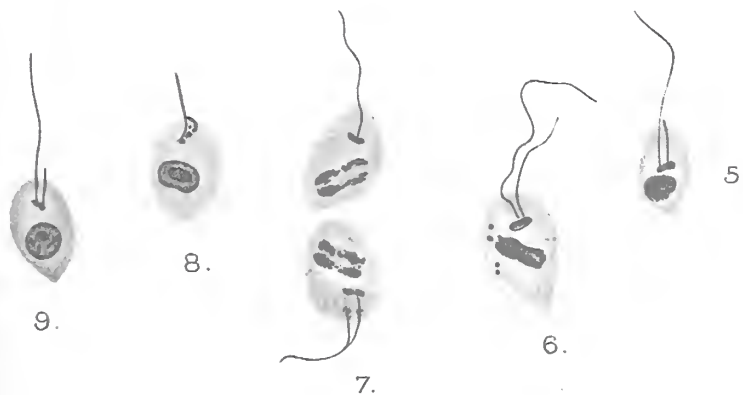
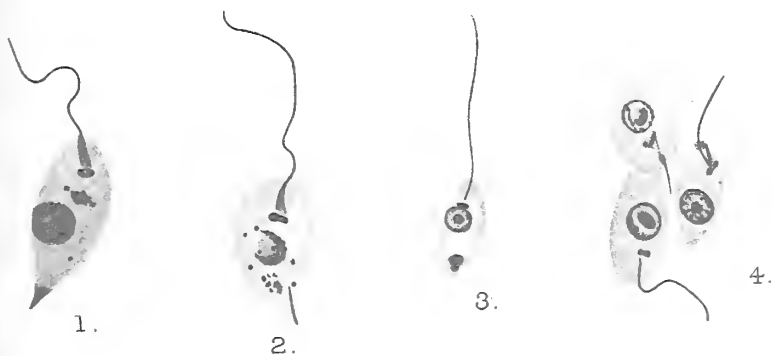
## PLATE 20.

Figs. 19-38.—Flagellated forms from cultures stained with iron-haematoxylin, magnified 2000.

Figs. 39-46.—Nuclei of the flagellated forms drawn at a magnification of 3000 diameters.



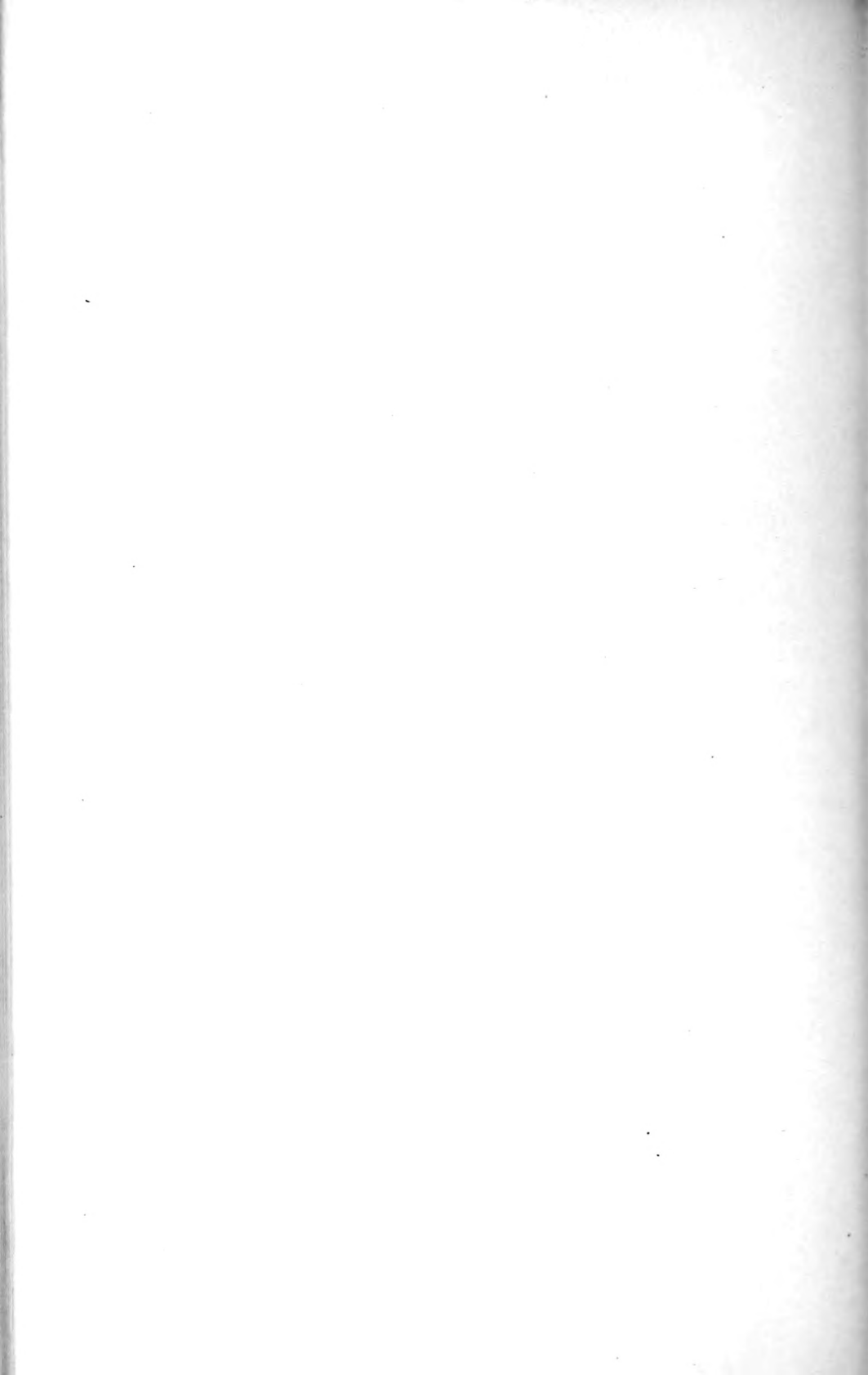


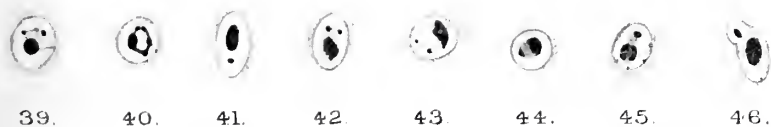
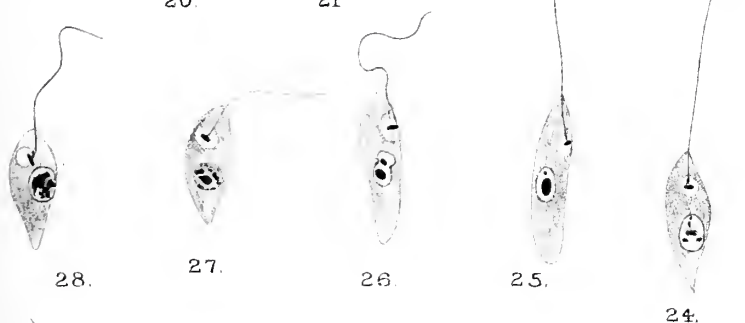


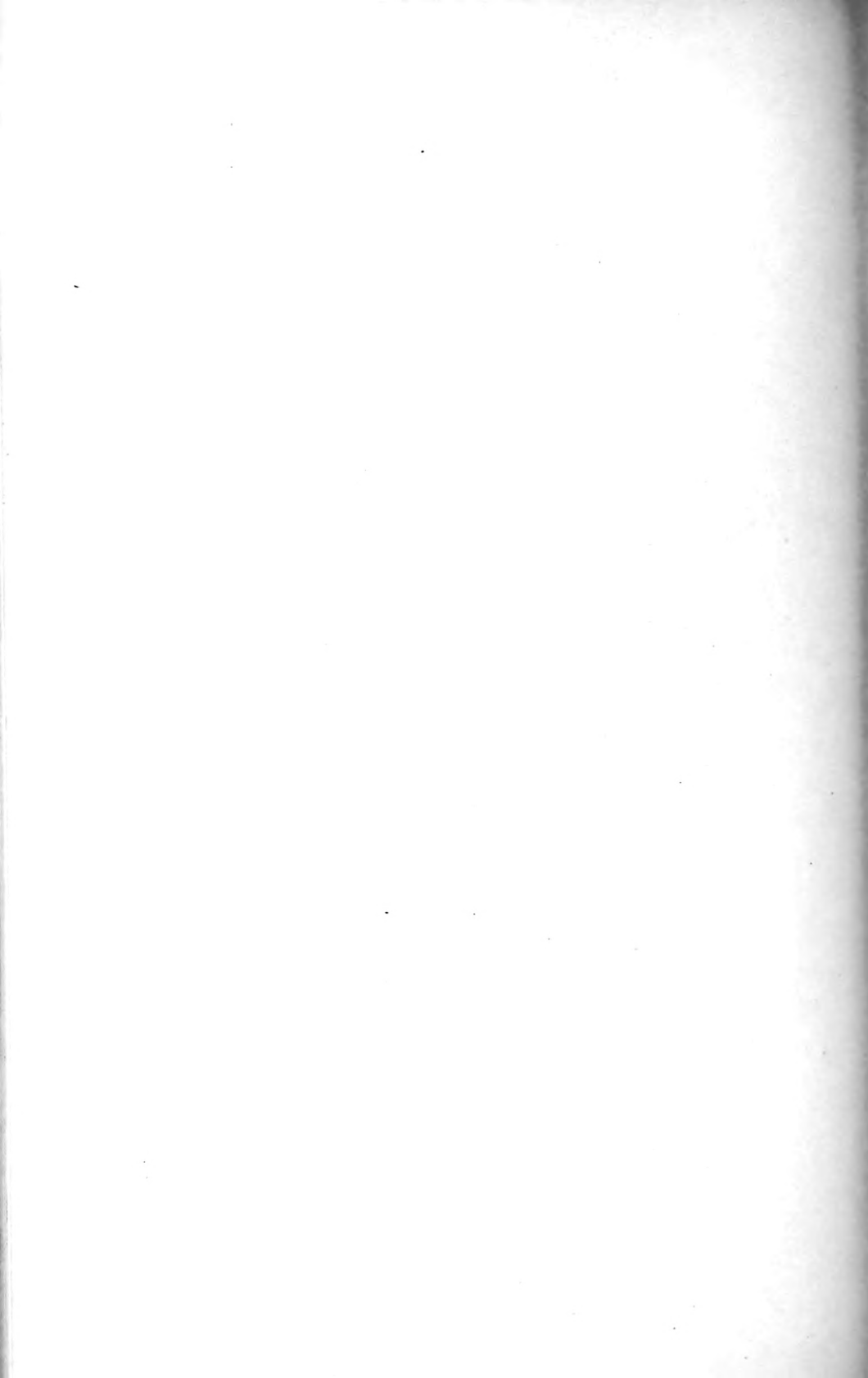
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LEISHMANIA INFANTUM







**The Transmission of Leishmaniosis by means of Cultures, and the Mechanism of the Natural Immunity in Rats and Guinea-pigs.**

By

**Dr. Arrigo Visentini.**

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With Plate 21.

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ATTEMPTS to transmit to animals the infection of Leishmania by means of cultural forms have not always had constant results up to the present.

Nicolle (1909) first inoculated dogs and monkeys (animals notoriously susceptible to leishmaniosis) with 1 c.c. of a culture, but without result; afterwards, however, in collaboration with Manceaux, he obtained positive results by inoculating *Macacus cynomolgus* and *M. sinicus*, reproducing the infection with the clinical picture of infantile leishmaniosis.

Novy (1908), by means of cultures obtained from Nicolle, had already succeeded in infecting dogs and afterwards other laboratory animals (?) by inoculating considerable quantities of the parasites, in the case of one dog about 270 cultures in fifty injections. Subsequently, however, Novy (1909) established the fact that a single injection of a suspension obtained from a score of culture-tubes is sufficient to produce leishmaniosis in dogs.

The rabbit has so far been proved to be susceptible only to corneal injection and to the forms occurring in the hæmatopoietic organs. Volpino, by scarification of the cornea with material obtained from a dog infected with experimental leishmaniosis, produced a keratitis similar, up to a certain

point, to that of experimental syphilis. In the circumscribed corneal lesions provoked by Volpino there were found typical Leishmanias, contained in the large mononuclears.

Basile and I have repeatedly inoculated large quantities of cultures in various ways, including the corneal method, without succeeding in our object. On the other hand, a single intra-peritoneal injection of a culture of *Leishmania* at the eighth transplantation, about fifteen days old and very rich in flagellate forms, was found by Franchini<sup>1</sup> (1911) to be sufficient to produce in a guinea-pig an infection with *Leishmania* which ran a course that might be termed acute. After six days the animal began to lose flesh and to show febrile symptoms; after twenty-six days the depression previously evident had become very marked. The guinea-pig was sacrificed before spontaneous death intervened. Its weight had diminished by almost half of the original amount. At the autopsy the liver and spleen were found to be enlarged; the marrow of the long bones was very abundant and of a reddish-brown colour; the supra-renal capsules were also enlarged. In the spleen, liver, bone-marrow, peripheral blood, supra-renal capsules and kidney Franchini found forms of *Leishmania* more or less numerous, but always extra-cellular, a state of things which, in the author's opinion, is in relation with the septicæmic type of the infection.

In the guinea-pig, rat and mouse, Laveran and Pettit (1909) have obtained slight infections of *Leishmania* localised constantly in the peritoneum as the result of intra-peritoneal, intra-hepatic, and even subcutaneous injections of an emulsion of organs of a dog infected with leishmaniosis. But by means of cultures infection was not obtained in the mouse, even after injections repeated many times and with increased doses. This was established by Delanö, who has thrown light on the mechanism of the natural immunity possessed by the mouse against the cultures, showing that in

<sup>1</sup> It is well to point out that Franchini, when he uses the name *L. donovani*, means (at least so I believe) the *Leishmania* which is the specific agent of kala-azar in Italy.



the peritoneum a very active phagocytosis of the flagellate forms takes place, so that after a short time they are broken up and completely destroyed.

Continuing previous researches left incomplete, I have again undertaken inoculations of guinea-pigs and white rats with cultures, having at my disposition besides the strain obtained by myself from a young patient, Rocca V—, of Bovalino Calabro, other strains also which I owe to the kindness of Mesnil and Jemma. While the cultures of the Pasteur Institute of Paris, originating from Tunis, had been kept alive for some years by successive transplantations, mine and those of Jemma were very recent and still in the first subcultures. The cultures used were at the height of their development, some in the first days of the subculture (3, 5 or 7 days), others older (10, 15 or 20 days) and others very old (1, 2 or 3 months). The object was to inject all the various forms which have been described in the cultures of *Leishmania*. The quantity injected was almost always 2 c.c., sometimes 4 c.c., for animals of medium size, guinea-pigs weighing about 300 gr. and young rats. In some cases 1 c.c. of the culture was injected, to try the effect of injecting the animals once, twice, thrice or four times. Twenty guinea-pigs and 10 rats were inoculated.

The method of injection was for the most part intra-peritoneal, and only in a few cases was the material of the culture injected under the skin or directly into the current of the circulation, by the vein of the tail of the rats or by means of heart-puncture—a method which seems to me preferable since it does not present excessive difficulties of technique.

Some of the animals died spontaneously, two guinea-pigs and two rats, in the course of the investigations, as happens often with these animals in the laboratory from causes extraneous to the experiments, and of these I made a rigorous examination in search of the *Leishmanias*; only one rat and one guinea-pig were lost without examination, and of these, of course, I have taken no account. The others were killed

by chloroform at various intervals of time from the first injection, from an hour and a quarter to ninety-three days after, and the autopsy was performed immediately. Both the guinea-pigs and the rats showed no signs of loss of flesh; some of them were even increased in weight. While they were still living, with a glass pipette having one end drawn out into a capillary tube I took from the peritoneal cavity a drop of yellowish liquid, slightly turbid, containing, as we shall see further on, numerous leucocytes, chiefly mononuclears.

The subsequent autopsy revealed nothing noteworthy; the internal organs, spleen, liver, kidneys, bone-marrow and lungs were not increased in volume or changed in appearance or in any way different from those of healthy animals. Of these organs I made smears which I fixed afterwards either in methyl alcohol or with osmic acid vapour and absolute alcohol and stained with Giemsa's stain. The most careful examination has never enabled me to discover a *Leishmania*, either in its usual form or in the flagellate leptomonad or other type, even when the autopsy followed close upon the inoculation of the cultures into the peritoneum.

Both of the blood and of the internal organs I have tried in each animal numerous cultures in blood-agar (method of Novy and Nicolle), and I have never obtained development of *Leishmanias*; the culture-tubes remained perfectly sterile, save for some rare exceptions (see below).

I think, therefore, that I am able to establish the point that guinea-pigs and rats possess a natural immunity against the cultural forms of the *Leishmania* of the Mediterranean basin, alike whether the strains are recently isolated or of long standing and whether the cultures are new or old subcultures.

The case of the infection of the guinea-pig obtained by Franchini with cultures remains, therefore, unique and must at least represent a somewhat rare event. It was a case perhaps of a single animal extraordinarily and exceptionally

receptive. Exceptional at least are the observations which Franchini describes. He found parasites in the peripheral blood and free in the plasma, and similarly all the forms of *Leishmania* found in the liver, spleen and bone-marrow were extra-cellular. It is, however, usual in Leishmanial infections, by general agreement of all observers, for the parasites to be always or nearly always contained in the protoplasm of the large mononuclears, and it has not yet been shown that they can occur free outside this their natural situation. Unfortunately the drawings which accompany the work of Franchini are so obscure and so little demonstrative that they do not permit one to judge whether one is dealing with *Leishmania*, the more so since the methods employed by the author are certainly not those the best adapted to obtain elective staining, but are even scarcely sufficient for diagnostic purposes, especially when the parasites, as in this case, are rather scarce.

In order to study rather more closely the mechanism of the natural immunity possessed by guinea-pigs and white rats, and at the same time to avoid any objection to the facts observed by me, I have carried out a double series of investigations, morphological and cultural, with the object of following step by step the fate of the flagellated forms in the organism of the experimental animals.

Having injected into the peritoneum 2 c.c. and sometimes 4 c.c. of a culture very rich in flagellated forms, I drew off every five minutes some peritoneal fluid with a sterile capillary glass tube and examined it fresh with the microscope, making at the same time some preparations for staining. The fact is quickly observed that the parasites undergo absorption rapidly by the leucocytes in the peritoneal cavity.

Prior, however, to describing the phenomenon in greater detail some points of technique may advantageously be dealt with. I have performed the experiment on a sufficiently large number of animals, guinea-pigs and rats, in such a way as to be able to perform, in each animal, only one or two punctures of the peritoneum, with the object of avoiding

those alterations which are produced by the simple fact of operative lesion. With regard to this I can confirm the observation of Delanoë for trypanosomes. When the puncture of the peritoneum is repeated several times, alteration and death of the *Leishmanias* take place by the probable extrusion of trypanolytic substances from the leucocytes into the plasma, while if the peritoneum be not punctured the flagellates that have remained free are preserved living and with normal structure, and in this condition are engulfed by the leucocytes.

Of the liquid extracted from the peritoneal cavity I have made preparations in the fresh condition and smears on slides which I have fixed while still wet in vapour of osmic acid for five seconds, and afterwards, when dry, in absolute alcohol for a quarter of an hour—a method of technique which also gives very good results for staining the flagellates in the cultures. The smears should be very thin, and it is often useful to follow the staining by very short differentiation in the solution of tannin according to Unna.

I have made examinations of the peritoneal liquid every five minutes after the injection up to two hours and then every half hour up to four hours, repeating the experiment in a fairly numerous series of animals. In the fresh state it is seen in the first few minutes after the injection of the culture that the number of the *Leishmanias*, truly enormous in 2-4 c.c. of a rich culture, is already to some extent diminished, and not by the fact of the greater dilution alone; very many flagellates are still free and very many are mobile, perhaps even more than in the cultural liquid, but it is not difficult to come upon others adhering or united to leucocytes, either by the posterior end or by the flagellum, preserving, however, in all cases a certain mobility. When they are completely engulfed in the leucocytes they are seen in its protoplasm as rounded bodies, but at this point it is easier to recognise them and to follow their modifications in the stained preparations. In the fresh state I have found in the guinea-pig some *Leishmanias* free and mobile up to an hour

or an hour and a half after the injection of 2 c.c. of culture into animals of 250 to 300 grammes in weight. In the rat I did not succeed in seeing free forms after an hour and a quarter.

In the stained preparations these various phenomena are very evident, and it is possible to follow step by step the various phases of alteration to which the *Leishmanias* are subjected.

One fact which deserves special emphasis is that the parasites are found always in the large mononuclears and in them alone, that is to say in just those elements which contain them in kala-azar and oriental sore. In the rare cases in which a true polynuclear is found apparently containing a *Leishmania*, the possibility cannot be excluded that it is simply superposed rather than engulfed. In the protoplasm of the mononuclears they become more rounded in form, preserving sometimes all their constituent parts and traces of the flagellum. Thus it is possible in some parts of the preparations to have the impression that it is a case of simple modification of the form of the *Leishmanias* and that they have found in the mononuclears a habitat adapted to them; but for the most part they present obvious alterations. The protoplasm becomes clear or turbid, the contours of the parasite disappear, the flagellum becomes thickened and breaks up; sometimes the trophonucleus or the kinetonucleus is wanting, or these bodies lose their sharp contours and their special characteristics and are reduced to shapeless masses of chromatin.

After fifteen to twenty minutes very many mononuclears are observed containing *Leishmanias* profoundly altered, represented after twenty to thirty minutes by remains of nuclei near which a portion of the flagellum is to be noted. The profound modifications of the phagocytosed parasites continue, and after two to three hours there are found only chromatinic granules with masses of protoplasm stained intensely in reddish violet, representing the last vestiges of the destroyed *Leishmanias*.

Even after thirty minutes up to an hour the first stages of the phagocytosis are still to be observed; mononuclears which contain forms greatly altered show others in process of being engulfed, while other flagellates still quite intact remain free and perfectly mobile in the serum, like that shown in Fig. 7, twenty-five minutes after the injection.

I have also made, as already stated, preparations of the peritoneal exudation of the animals sacrificed at various periods up to ninety-three days after the injection of the cultures and I have never seen *Leishmanias* or remains of them in the mononuclears, although I have directed special attention to this point, because Laveran and Mesnil have observed the peritoneal infection up to fifty-nine days after the injection of emulsion of organs of a dog infected with experimental leishmaniosis and have obtained subcultures of this fluid.

Contemporaneously with the examination of the peritoneal fluid I have repeatedly made an examination of the peripheral blood, and, in animals sacrificed, I have made smears of the various organs; in spite of the most patient investigation I have never met with any form of *Leishmania* or any flagellate parasite in my numerous preparations.

The facts described permit me to assert that we are dealing with a process of phagocytosis pure and simple; it cannot be admitted that the flagellate leptomonad forms are transformed into *Leishmania*-forms and as such continue to live in the mononuclears, producing a slight infection localised in the peritoneum, of the type of that obtained by Laveran and Pettit, because we are witnesses here of a gradual alteration and destruction of the parasites by a process which leads to the final disruption of all remains of the injected Protozoa. It is scarcely necessary to add that in the forms engulfed every sign is lacking of a process of multiplication. Moreover the results of attempts to make cultures confirm fully this interpretation, and give a proof of it which I think can be regarded as absolute. In every animal killed with chloroform I have made cultures in blood-

agar according to Novy-Nicolle of blood taken aseptically from the heart, spleen, liver, bone-marrow, and in some cases of the peritoneal fluid. The results were constantly negative except with the peritoneal fluid a short time after the injection of the culture, when it is still possible to find by microscopic examination some *Leishmanias* living and mobile. Thus after an hour and a quarter in one rat I was able to obtain a new culture from the peritoneum, while after two hours the result has always been negative.

In complete accord with the result of the histological examination I have never obtained development of flagellates in a culture from the blood or from the internal organs, not even by injecting into the peritoneal cavity or directly into the circulation 4 c.c. of cultural fluid very rich in *Leishmanias*. These flagellate Protozoa would not, therefore, pass beyond the peritoneal barrier in the guinea-pig and the rat.

I have stated above that I have injected the cultures of *Leishmania* directly into the blood in some animals. Although my investigations may not be sufficiently numerous, being limited to one guinea-pig and one rat alone, I have noted in the blood immediately after the injection a marked leucocytosis with prevalence of mononuclears, which reached its maximum degree after five to ten minutes, was maintained for half an hour, and then diminished gradually. In these cases also I was never able, even soon after the injection, to find flagellate *Leishmanias* in the blood, nor have I seen phagocytosed forms. The animal has not contracted any infection.

#### SUMMARY.

Guinea-pigs and rats possess natural immunity to a high degree against the *Leishmania* of the Mediterranean basin in the flagellate stage in cultures on blood-agar (method of Novy-McNeal-Nicolle).

This immunity is exclusively of phagocytic nature. The Protozoa injected into the peritoneum become rapidly en-



gulfed by the leucocytes, the mononuclears exclusively, and undergo gradual and progressive alterations, ending in their complete destruction, so that by the end of an hour or an hour and a half after the injection of 2 c.c. of a culture in full development, free flagellates are no longer to be found in the serum, and after two or three hours even the last vestiges of them are almost destroyed in the protoplasm of the phagocytes.

The Protozoa do not pass beyond the barrier of the peritoneum and do not find their way in this manner into the blood or the internal organs.

These data of observation receive full confirmation in the results of the cultures. It is possible, in fact, to obtain sub-cultures from the peritoneal fluid up to about one hour after the injection of 2 c.c. of liquid of condensation very rich in Leishmanias, but not beyond this period, nor are sub-cultures obtained from the blood or organs of the animals in experiment.

I desire to express my gratitude to Dr. Martin for having received me with so much kindness and hospitality at the Lister Institute of Preventive Medicine, London, of which he is the Director, and for having provided me liberally with the means of research; I also thank Dr. Bayon warmly for assisting me in every way during my stay in this Institute.

LISTER INSTITUTE OF PREVENTIVE MEDICINE,

LONDON.

May 25th, 1912.

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## DESCRIPTION OF PLATE 21

(From smears of peritoneal fluid),

Illustrating Dr. Arrigo Visentini's paper on "The Transmission of Leishmaniosis by means of Cultures, and the Mechanism of the Natural Immunity in Rats and Guinea-pigs."

[The figures are drawn with the camera lucida at a magnification of 2000 diameters.]

Fig. 1.—Five minutes after the injection of a culture of *Leishmania*, showing one parasite absorbed, and another in process of absorption, by a mononuclear leucocyte.

Fig. 2.—Ten minutes after injection; a leptomonad form absorbed, another held fast by its flagellum.

Fig. 3.—Thirty minutes after injection; a mononuclear containing three parasites, in process of destruction, and in the act of absorbing a fourth.

Figs. 4 and 5.—Fifteen minutes after injection; mononuclears containing Leishmanias in process of destruction.

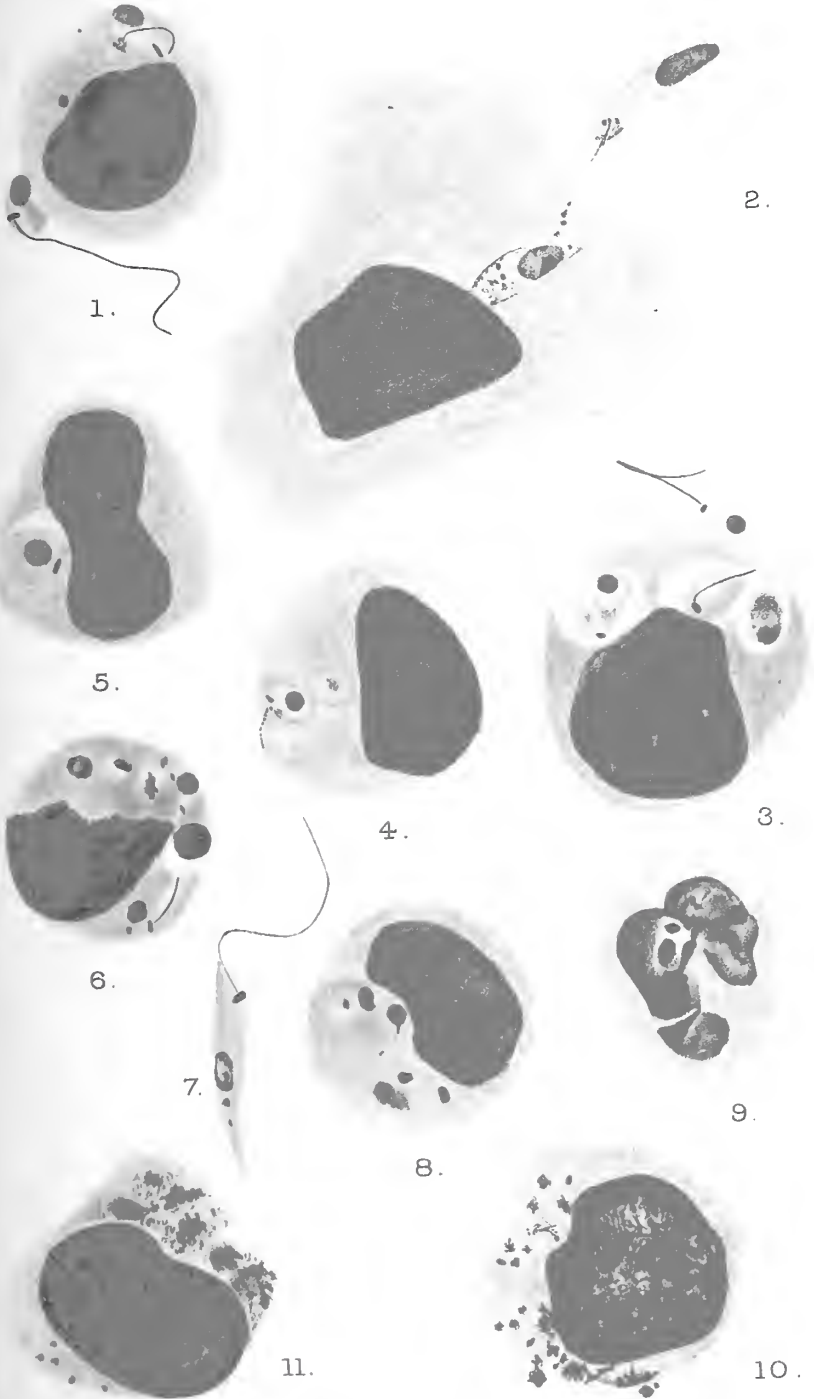
Fig. 6.—Twenty minutes after injection; mononuclear containing the remains of several parasites.

Fig. 7.—Free Leptomonas-form twenty-five minutes after injection.

Figs. 8 and 9.—One hour and a quarter after injection; a mononuclear containing remains of several parasites and a polymorphonuclear apparently containing (?) a parasite.

Fig. 10.—Two hours after injection; mononuclear containing the last vestiges of several parasites.

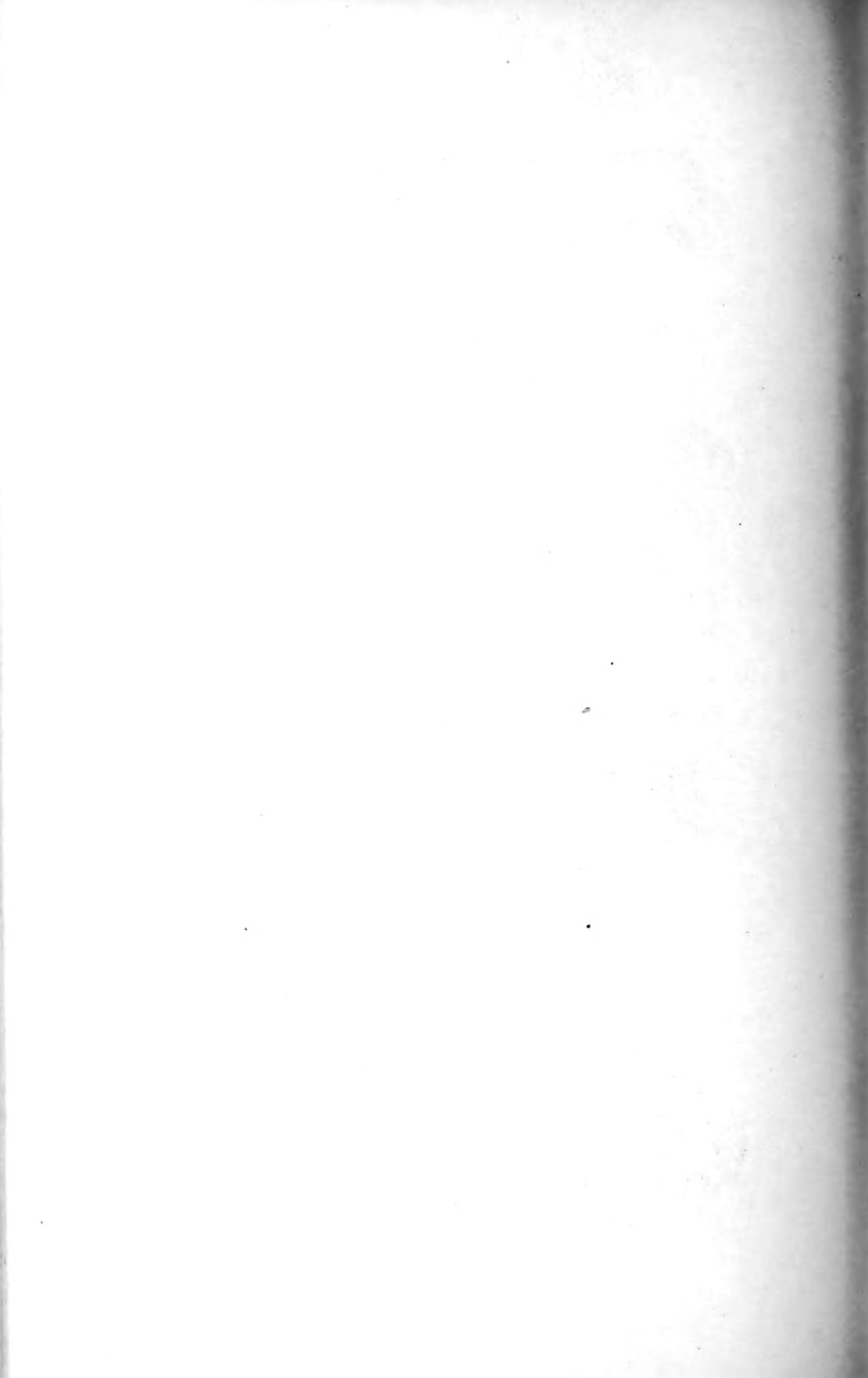
Fig. 11.—Three hours after injection; as the last.



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# PHAGOCYTOSIS OF LEISHMANIA



**Herpyllobius arcticus.**

By

**Kathleen Haddon.**

With Plate 22 and 4 Text-figures.

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## I. INTRODUCTION.

AMONG parasitic copepods *Herpyllobius* is remarkable for the extreme degeneracy attained by the adult male and female, both of which are entirely devoid of appendages. The female has a globular body 2 to 4 mm. long, which bears two large egg-sacs. It is attached to its host, a polychæte worm, by means of a chitinous fixing organ, through this there protrudes into the worm a mass of vacuolated

tissue, recalling in its structure and nutritive function the root system of the parasitic *Rhizocephala* amongst the Cirripedes. It seems hardly likely that an endoparasitic stage is included in its life-history, but of this we are entirely ignorant. The adult males have a sac-like body, which remains enclosed in the last larval skin; they live attached to the body of the female in the region of the genital apertures.

Steenstrup and Lütken (12), who first described this species, *Herpyllobius arcticus*, found it parasitic on certain polychaete worms from Greenland, and recognised that the body consisted of two parts, the external body and the part within the host, joined together by a thin stalk. The external body was rounded, and bore a pair of large egg-sacs, whilst the internal portion they believed to vary in shape according to the amount of space available within the worm. They found no males.

Two years later Krøyer (7) published an account of a parasite, which he called *Silenium polynæ*s, found infecting *Polynæ cirrata* in Greenland. He only described the portion of the parasite external to its host, but he was more fortunate than Steenstrup and Lütken, in that he found the males, four or five in number, attached near the egg-sacs of the female; they were minute Cyclops-like forms, about .13 mm. long and half as broad, the anterior end of the body being drawn out and inserted firmly into the body of the female. He gave some details of the appearance of both male and female, which will be dealt with later in discussing the anatomy of these forms.

In 1874 Claus (1) published a paper, in which he placed *Silenium* among the *Lernæopodidæ*, and suggested that it was identical with the *Herpyllobius* described by Steenstrup and Lütken. Further light was thrown on the systematic position of this animal by Levisen (9), who, in an account of some parasitic copepods, gave a summary of the generic characters of *Herpyllobius* and the specific characters of the two species then known, *H. arcticus* (Stp. and Ltk.)



and *H. crassirostris* (Sars). In this paper Levinsen also dealt with the different theories concerning the significance of the portion of the parasite inside the worm, and gave a concise account of his own observations on the subject.

Giard and Bonnier (2) in 1893 published the results of their observations on *Sphæronella*, and suggested including the *Herpyllobiinae* as a sub-family equivalent to the *Choniostomatinae*, under the heading of *Sphæronellidae*. This classification was not acceptable to Hansen (4), who criticised their conclusions in his work on the *Choniostomatidae*, and objected to *Herpyllobius* being linked with that family on the grounds of extreme structural differences exhibited by the two forms.

In 1900 Jensen published an account of *Herpyllobius* and *Rhizorhina* (6), and later on in the same year Hansen (5) wrote a paper on the same subject. Both these observers were able to make a minute investigation of these forms, with the result that their structure was worked out with greater detail than had before been possible.

As will be seen from this short account of the literature on *Herpyllobius*, almost all that has been written on the subject is in Danish, and hence not easily available. In the summer of 1911 Mr. F. A. Potts, of Trinity Hall, Cambridge, collected some specimens of this interesting parasite from Departure Bay (Vancouver Island), Victoria, and San Juan Island, in Puget Sound, and on his return to England very kindly gave them to me to describe. This provided me with opportunity of writing an account of the animal in what I trust may be a more accessible form for English readers than at present exists.

I should like here to thank Mr. Potts for the help he has given me in writing this paper, and also Mr. H. M. Chadwick, of Clare College, and Miss Lehmann, of Newnham College, Cambridge, for their assistance in translating the Danish papers, and to acknowledge the kindness of Dr. H. J. Hansen in sending me his paper on *Herpyllobius*, and of Dr. W. T. Calman in reading my proofs.

I should also like to thank the trustees of the Bathurst Fund, Newnham College, for a grant awarded to me in the summer of 1911.

## II. MATERIAL.

The copepods were found attached to specimens of the polychæte worm *Harmothoë*, and all were fixed in corrosive and acetic and preserved in 70 per cent. alcohol.

There were seven worms and ten parasites, distributed as follows: three worms had one parasite each on the head, two worms had one parasite each on a parapodium, one worm had a parasite on the head and another on a parapodium, and one worm had three parasites on the head. The parasites were always attached to the dorsal surface of the host.

## III. ANATOMY.

### A. The Female.

1. External Appearance.—The female is entirely devoid of appendages, and the portion outside the host is globular in shape, except for swellings at the bases of the egg-sacs; in some of the smaller forms, which are probably young as their egg-sacs are not developed, there is a tendency for the body to be flattened ventrally. Levinsen (9) describes *Herpyllobius arcticus* as being triangular in outline and laterally compressed, but Kröyer's description (7) of a sac-like body seems to tally better with this form. The body is smooth and of a white or yellowish colour, and some specimens show the slight longitudinal grooves, mentioned by Steenstrup and Lütken (12), which, as they were observed in fresh specimens by Mr. Potts, cannot be due to shrinkage in spirit, as was suggested by these observers.

The length of this part of the parasite is from 1 to 1·3 mm.; Levinsen (9), in his summary of the specific characters of *H. arcticus*, gives the length as 1·5 to 2 mm., but on p. 367 he gives lengths varying from 0·6 to 2 mm., so that there is

evidently a good deal of variation. This portion of *H. crassirostris*, according to the same observer, is  $\frac{2}{3}$  mm. long, while Hansen (3) describes *H. affinis* as being 3 to 4 mm. in length.

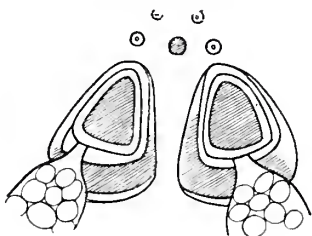
The paired egg-sacs naturally vary a good deal in size according to the stage of development, and they may be larger than the body; they are rounded or sausage-shaped, and in one individual flattened and leaf-like. Each egg-sac is borne on a strong chitinous swelling, which is somewhat shield-shaped, with the point directed upwards; these two structures, according to Levinsen, consist of concentric rings of chitin (Text-fig. 1, and Pl. 22, fig. 2). The eggs, owing to their being tightly packed, are polygonal in outline. Above the bases of the egg-sacs are some small apertures with chitinous edges. I could not make out the arrangement of these as they only became apparent in my specimens after they were sectioned. Levinsen, however, describes and figures them as four in number and forming an arc, in the centre of which is a small process previously described by Steenstrup and Lütken (Text-fig. 1). Jensen (6) figures two more similar apertures, one at the apex of each chitinous boss. On the ventral surface are two small chitinous bodies which Kröyer suggested might be lenses. This, however, seems unlikely, as an examination of the sections shows no trace of any lens-like structure in this, or any other region. Kröyer was probably misled by his assumption that the whole of the parasite was external to the worm, whereas it is now generally accepted that the visible portion is hardly one half of the whole animal. The diminutive males live attached to this posterior region close to the egg-sacs of the females (Pl. 22, fig. 2).

The external portion of the parasite is joined to the part lying within the host by a narrow stalk, on either side of which some specimens show a light-coloured area. Just underneath the skin of the host the stalk becomes chitinised and expands into a hold-fast, which is collar-shaped with serrated edges (Pl. 22, figs. 5 and 7).

2. Part embedded in the host.—This part of the

parasite is generally called the "anterior portion" or "proboscis," presumably because other parasitic copepods, such as *Lernæa* and *Pennella*, are attached by what is obviously the anterior end, and it is not likely that *Herpyllobius* would depart from the general rule, especially as the males are attached anteriorly; it is also evident that the external portion must be posterior because of the presence of the egg-sacs. Owing, however, to the extreme degeneracy attained by this animal, the "anterior portion" has lost any characteristic structure it may have had and become merely absorptive, thus recalling a similar state exhibited by the

TEXT-FIG. 1.



(After Levinsen.)

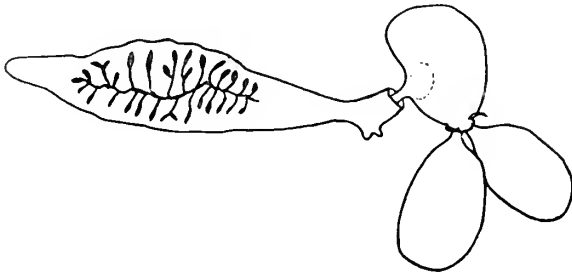
Rhizocephalan Cirripedes; for this reason I propose to speak of this portion of the parasite as the "root system."

This was not visible in my specimens until the worms were sectioned. Levinsen (9), however, was able to see it within the host by simply removing the elytra of the worm. He described it as a thin red streak lying parallel to the worm's gut, and on removing the worm's skin it appeared as a long, narrow, tongue-shaped body lying free beside, or underneath the pharynx of its host; posteriorly it emerged from the worm's head and was continuous through the stalk with the external part (Text-fig. 2). The length of the root system of the parasite according to this observer was 4 to 5 mm., the breadth 1 mm., and the thickness  $\frac{1}{3}$  to  $\frac{1}{2}$  mm.; the broadest part was just behind the middle, and posterior to this it tapered, broadening again just before the stalk, where

it gave off two small processes. Along almost the whole length there were red dendritic markings, but no cell boundaries were visible: in spirit specimens some lacunæ showed.

This structure has been the source of some controversy; Steenstrup and Lütken (12) first described it as part of the parasite, but Krøyer (7) some years later regarded the chitinous "sucker" as the end of the parasite, and Schiödte (11) agreed with Krøyer that no integral part of the parasite was inside the worm. He recognised two terms used by different observers, "opsvulnet" and "tungenadannet,"

TEXT-FIG. 2.



(After Levinsen.)

and explained their meaning by translating the first as a "tumour" formed by the worm, and the second as a "tongue-shaped body" hanging from the sucker and caused by some excretion from the parasite coagulated by spirit. Sars (10) also agreed with Krøyer, but as his specimen of *H. crassirostris* was still attached to the host, the part in question would not show. Claus (1), describing *H. arcticus*, thought that the front part was pathological.

Levinson (9) then went thoroughly into the question, and found by making a cut through the stalk that the cuticle surrounding the root system appeared to be continuous with that of the stalk. He also made careful measurements of the two portions of fourteen individuals, and obtained the following

results: the top figures giving the length in mm. of the roots, whilst those below give that of the external portion:

$\frac{5}{2}$	$\frac{5}{2}$	$\frac{4.5}{1.5}$	$\frac{4.5}{1.5}$	$\frac{4}{2}$	$\frac{4}{1.6}$	$\frac{4}{1.6}$	$\frac{4}{1.5}$	$\frac{4}{1.3}$	$\frac{3.6}{1.6}$	$\frac{3.5}{1.5}$	$\frac{3.3}{1.3}$	$\frac{3}{1}$	$\frac{1.5}{0.6}$
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showing that the root system is from two to three times as long as the part outside the worm. He therefore concluded that there is a definite body inside the worm which is connected with the external parasite, and is either part of the parasite or a pathological formation caused by it, but that the former is the case as this structure is always the same shape, it lies free in the worm's body, and is proportional in size to the external body. Other observers—Hansen (3) and Jensen (6)—also figure the roots as being in direct communication with the external part of the parasite.

The suggestion that the internal part of the parasite had a nutritive function was put forward by Levinsen (9), but this question will be dealt with in the next section, together with a description of its structure.

3. Microscopical Structure.—The first specimens cut were unfortunately detached from their host, as I did not then know that any part of the parasite was within the worm; subsequently the worm was cut at the same time as the parasite. The specimens were first stained in paracarmine, dehydrated and cleared, and then as much as possible of their structure was studied and drawn before they were sectioned; after being cut the sections were stained on the slide in hæmalum.

(a) External Part.—Examined as a solid object the anatomy of this portion of *Herpyllobius* is not easy to make out. The body is always covered by thick structureless cuticle from which the internal organs frequently shrink away. In most forms (Pl. 22, fig. 3) the body is almost filled with a mass of eggs, which may show clear spaces running through it, all converging at the point where the stalk arises, in which place there is often an opaque structure which runs down the stalk and may extend up the clear spaces. The strong chitinous buttresses that support the egg-sacs are well supplied

with muscles. The external skin is continuous down the stalk, at the end of which it becomes chitinous, and, turning up, forms a collar (Kröyer's "sucker"), which is embedded in the tissue of the host, and acts as a hold-fast. Some of the internal organs run down the stalk and are directly continuous with those of the root system.

When cut in sections and examined under the microscope, the cuticle of these animals appears as a thick layer, slightly chitinous and traversed by a number of fine wavy fibres running parallel to the surface; externally it is bounded by a thin layer which stains more deeply than the rest of the skin.

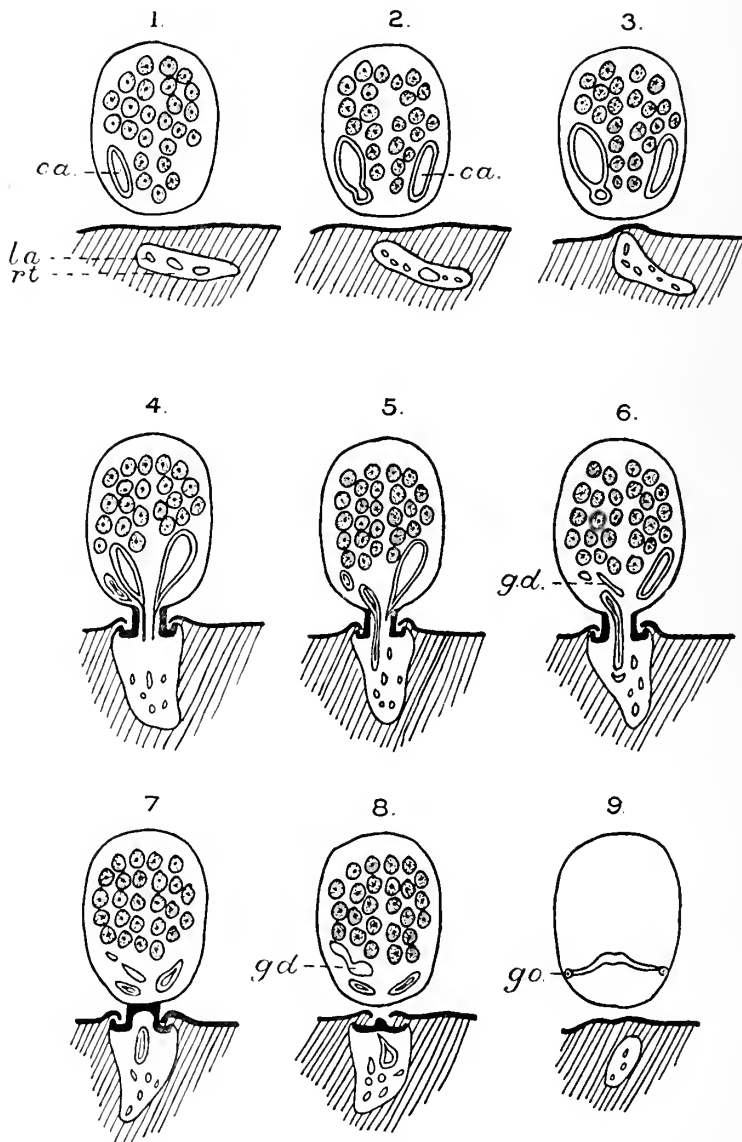
Lying beneath the cuticle and at intervals sending up fine processes into it is a thin layer (Pl. 22, fig. 6, *hyp.*) with nuclei scattered along it; it usually appears to be without any definite structure except in one case (Pl. 22, fig. 4, *hyp.*), when in longitudinal section this layer appears to be composed of epithelial cells; longitudinal sections of other individuals, however, do not show this structure. This layer probably represents the hypodermis, but it shows a tendency to shrink away from the cuticle and applies itself closely to the internal organs; the processes running into the cuticle may be caused by a folding of the sections. In the anterior region of this external portion some sections show canal-like openings through the skin, but there is no alteration in the tissues below them, so it is likely that these are also due to folding caused by shrinkage.

Posteriorly there are several chitin-lined apertures (Text-fig. 1), according to Jensen (6) six in number, to which, he says, are attached the males, although they are not the genital openings. Opening into each of these there is a peculiar gland, which he figures (6, Tab. ii, fig. 12). I have examined these glands in my own sections and can find no other opening except through these apertures.

The genital openings are large slits placed on the end of the chitinous supports of the egg-sacs, and from them a duct may be seen running to the inside of the animal where it



TEXT-FIG. 3.



Series of sections through a normal female of *Herpyllobius aeticeus*, showing external portion and root system (*rt.*). The tissues of the worm are shaded. *ca.* Canal system of external portion. *g. d.* Genital duct. *g. o.* Genital opening. *la.* Lacunar spaces in root. *rt.* Root.

becomes confused with the other spaces (Text-fig. 3, and Pl. 22, fig. 4, *g. d.*).

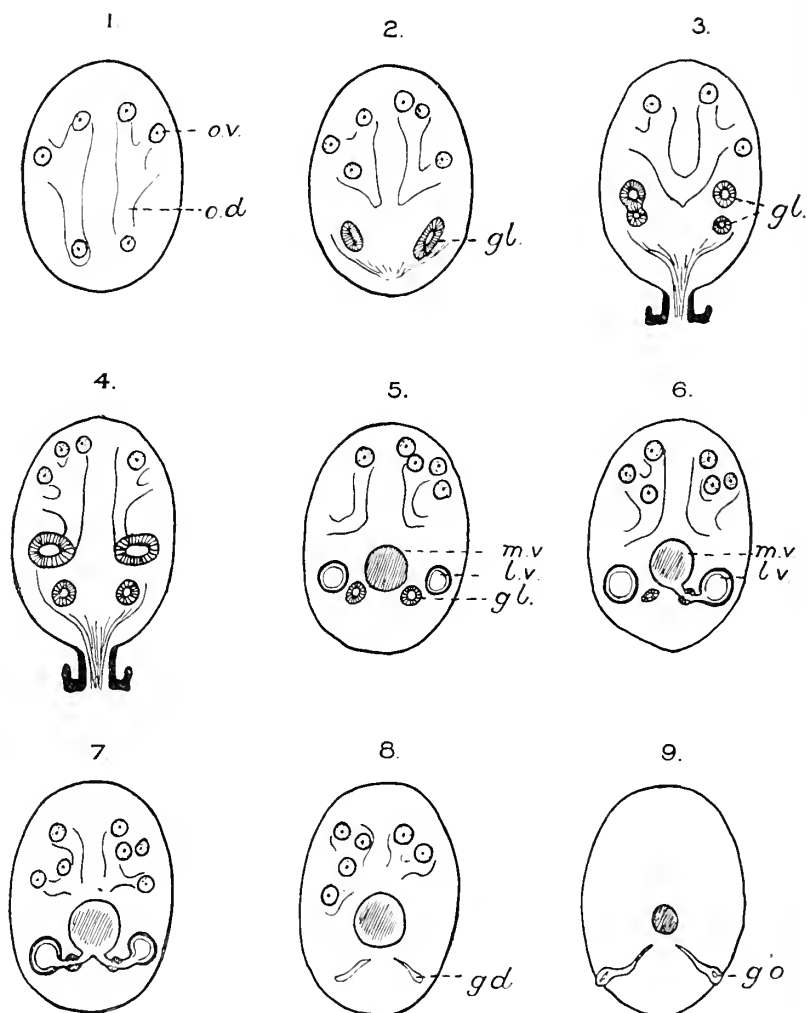
The body of the animal is frequently filled with eggs, which tend to be arranged in groups surrounded by strands of fibrous tissue which radiate from a central mass (Pl. 22, fig. 4, *f. b.*). Running from the stalk along each side of the animal there is a canal (*ca.*) surrounded by thick walls, and drawn out so as to extend down the stalk and become continuous with the lacunar system of the roots.

One specimen, however (Pl. 22, fig. 5), differed widely from the rest; it was rather small, and its egg-sacs were just beginning to form. It was less opaque than the others, so that the internal structure was more easily seen. The body was well filled with eggs, and running among them was a series of canals, presumably the oviducts (*o. d.*), which converged towards the middle of the animal, and apparently opened on either side into a thick-walled duct (*gl.*) which would homologise with the glandular portion of the oviduct described by Marcus Hartog<sup>1</sup> in *Cyclops* as secreting the cement by which the eggs are agglutinated together when expelled. Each of these ducts was bent into a **U**-shape and opened into, or near, a spherical vesicle (*l. v.*), which, in its turn, led to the exterior by a canal running out to the chitinous support of the egg-sac of that side, and opening near its apex. From this there extended an extremely small egg-sac, containing but few eggs. Between the glandular ends of the two oviducts there lay a large globular body (*m. v.*) containing a darkly staining mass, and having no obvious connection with the rest of the system; this, I thought, might be the spermatheca, as it lay not far from the place to which males were attached in other specimens. None of these structures extended down the stalk, the lumen of which was completely filled with non-vacuolated tissue.

An examination of sections made from this specimen also shows that its anatomy is very different from that of other

<sup>1</sup> Hartog, M., "The Morphology of *Cyclops* and the Relations of the Copepoda," 'Trans. Linn. Soc. Zool.,' v, 1888, pp. 1-46.

TEXT-FIG. 4.



Series of sections through another female *Herpyllobius arcticus*, showing the arrangement of the genital ducts. *g.d.* Genital duct. *gl.* Glandular portion of oviduct. *g.o.* Genital opening. *l.v.* Lateral vesicle. *m.v.* Median vesicle. *o.d.* Oviduct. *ov.* Ovum.

forms (Text-fig. 4). Directly beneath the hypodermis are a number of eggs (*o. v.*) lying in blind diverticulæ of the oviducts (*o. d.*); about the centre of the animal these converge into a single canal, which almost immediately divides again into the two glandular ducts (*gl. d.*) mentioned before, which run forward for a little way and then bend sharply back on themselves, so that each appears double in a considerable number of sections. Midway between these two tubes is a hollow sphere (*m. v.*) partially filled with a mass of tissue, frayed out at the edges into what, under an oil-immersion lens, looks remarkably like spermatozoa, and I have thought that this was a single spermatheca, such as is found in some Copepods. Such an interpretation is at variance with Jensen's statement that large spermatophores are found in the male and fixed by means of a cement to the upper end of the long genital aperture of the female (see his figure, (6), Tab. ii, fig. 11A, *hls.*), and I give it with reserve.

At the lower end of this median vesicle there is, at either side, a tube with swollen lips, which leads outwards to another vesicle (*l. v.*), which is smaller than the median one, surrounded by thick walls, and apparently empty. Into each of the ducts, from the median to the lateral vesicles, there opens the glandular oviduct, and from it there runs posteriorly another duct (*g. d.*), which opens to the exterior by a long slit, the genital aperture (*g. o.*) at the end of each of the chitinous bosses; these openings naturally only show when no egg-sacs are present.

Even in the sections there was no trace of any canal running down the stalk.

(b) Stalk and Root System.—As mentioned above, the skin of the external portion may be traced down the stalk to the chitinous hold-fast; this has a serrated edge to which are attached strands of muscle-fibres (Pl. 22, fig. 7, *m. f.*). Down the lumen of the stalk the tissues of the two parts of the copepod may be seen in direct communication, whilst the lacunar system of the roots may also be shown to communicate with certain spaces in the external portion, as noted before.

This last fact would seem to prove that Steenstrup (12) was right in his statement that the "Fordøjelsesvej" (literally "way of digestion") goes through the stalk. As Hansen (5) points out, he avoids calling this system the gut, for in a degenerate form like this no definite gut can be traced. Sections in this region show that these canals have very thick walls, which might be the remnants of gut epithelium—an additional reason for supposing that these spaces have a nutritive function.

The minute structure of the portion of the parasite embedded in the host has been the subject of a good deal of dispute. According to Leviusen (9), it is surrounded by a thin cuticle which appears to arise in the region of the sucker, and Jensen (6) describes this cuticle as being a direct continuation of the sucker, and pressed against the bent-up portion of it; Hansen (5) agrees that this suggestion is plausible, but points out that Jensen does not draw this cuticle separate from the skin he describes as formed by the worm round the parasite (6) (fig. 10). Jansen further suggests that it is this membrane, formed by the worm, that Leviusen described as the cuticle of the parasite, but Jensen considers it to belong to the host on the grounds that a section shows that the cells forming it lie to the outside, that is on the side remote from the parasite. In this connection he refers to Giard and Bonnier,<sup>1</sup> who describe a similar formation among crabs infected by *Entoniscans*. Hansen, however, does not believe that there is any other skin than that belonging to the parasite, and fails to find any trace of a membrane arising from the worm. He believes that an apparent membrane is due to the tissues of the worm being pressed against the roots of the parasite causing a certain amount of degeneration, and compares it with *Rhizorhina*,<sup>2</sup> the roots of which pass into the gills of *Ampelisca* with no trace of membrane round them.

A careful study of my sections leads me to believe that

<sup>1</sup> Giard and Bonnier, 'Contributions à l'étude des Bopyriens,' 1887, p. 97.

<sup>2</sup> Hansen, H. J., "Rhizorhina ampeliscæ," 'Ent. Meddel.' IIIb, H. 5, 1892, p. 207.

Jensen is right in stating that a membrane formed by the worm is present. In cases where the parasite and host have been cut together the epidermis of the worm folds in over the hold-fast (Pl. 22, fig. 7, *ep.*), and although the actual connection is not evident, the membrane (*mb.*) surrounding the roots seems to be a part of this. In sections of a whole worm from which the parasite had become detached the epidermis of the host (Pl. 22, fig. 8, *ep.*), although broken at the critical spot, is probably continuous with the membrane (*mb.*). This membrane may be traced down the worm as far as the roots of the parasite extend, although all the proximal part of the root system was withdrawn when the copepod was removed. A section lower down the worm (Pl. 22, fig. 9) shows the distal end of the roots of *Herpyllobius* surrounded at some distance by a well-marked membrane (*mb.*), and sections of another worm infested with three parasites show the root system of each surrounded by a membrane, which is always separated by some distance from the tissue of the root, and therefore is not likely to belong to it, as the root itself does not appear to be at all shrunken. Giard and Bonnier<sup>1</sup> state that in the case of crabs the parasitic Eutoniscan is always entirely surrounded by a thin transparent membrane, except at one spot which is the opening to the invagination caused by the penetration of the young Eutoniscan into its host; this membrane presents the same histological structure as the hypodermis of the crab. As a like process is probably effected by *Herpyllobius* it may then be described as an ectoparasite.

As to the cuticle of the roots themselves, I am inclined to think that if this is present it is extremely thin, and only evident in the region of the hold-fast (Pl. 22, fig. 7, *cut.*<sup>1</sup>); in sections of the worm with the parasite roots, these are not surrounded by any well-marked cuticle (Pl. 22, fig. 9).

The tissue of the root system is fibrous and does not show any definite cell boundaries; these fibres are strongly developed round the hold-fast, to the serrated lower edges of

<sup>1</sup> Loc. cit.

which they are attached (Pl. 22, fig. 7, *mf.*). All through this mass of tissue there is a branching system of lacunæ surrounded by a fairly definite lining of cells; the main one (*ca.*) runs up the stalk, and dividing into two is continued as a pair of lateral spaces in the external body; these, as mentioned above, have thick walls, which are also characteristic of this main canal for a short way along the root.

#### B. The Male.

The males are diminutive, and live attached to the female in the region of the egg-sacs; there are usually two or three to each female, and according to Jensen (6) they fix themselves to the openings of the peculiar glands mentioned before as occurring in this region. As, however, these glands are only six in number at the most, it is difficult to see how they could accommodate the twenty males found by Hansen (3) on one female of *H. affinis*.

The cephalic segment, which occupies about one half of the body (Pl. 22, fig. 10) has a thoracic segment fused to it, for in addition to a pair of three-jointed antennæ it bears a pair of maxillipeds; these are four-jointed, the last joint being hooked and serving for attachment to the female. There are three thoracic segments, each bearing a pair of biramous swimming legs; the first two pairs are alike, each consisting of two basal joints, a one-jointed inner ramus and a two-jointed outer one, each bearing bristles. The third pair resembles the first two except in having a one-jointed outer ramus. The abdomen consists of three narrow segments, the first two being about equal in size, whilst the third is about twice their length and bears a forked telson, each prong composed of two joints and bearing three stout bristles.

According to Jensen, however, this chitinous investment is merely the last larval skin of the male, the adult being sac-like and enclosed, all except the anterior end, in the skin; the anterior end is drawn out and is embedded in the female, being kept in position by a rough surface. The two genital openings



(*go.*) lie side by side at the base of the drawn-out anterior end, and near its apex there appears to be another opening (*m.*) which is not so obvious as the genital apertures, but its position is indicated by a strand of tissue running up to it. This is the "mouth" described by Jensen, and he further finds a pair of mandibles and two pairs of small processes, possibly rudiments of appendages, on the hinder pair of which there open a pair of glands which secrete cement for fixing the male to the female. My specimens, however, showed no mandibles or processes, and their existence is denied by Hansen (5), who furthermore declares that there is no mouth opening at all; I am, however, inclined to believe that a mouth is present, although it is degenerate and probably functionless.

The body part of most of the males is chiefly occupied by two large spermatophores (*sp.*) oblong, in shape and provided with long necks (*sp. d.*), which emerge through the genital opening; these are evidently for insertion into the female and may be of great length; in one of my specimens they are twice as long as the whole animal. A similar structure has been described by Hansen in the allied genus *Rhizorhina*.<sup>1</sup> Jensen believes that the cement for the spermatophores is derived from two large glands occupying all the ventral and part of the dorsal surface anterior to the single testis (6, tab. ii); none of my specimens, however, showed this gland; and Hansen considers that the cement is derived from the vas deferens. The spermatophores, according to Jensen, are shed from the body of the male, and he figures them (6, fig. 11A) attached to the female close to the genital openings; they were not to be found on any of my specimens.

The anterior position of the genital openings of the male is an unusual occurrence among crustacea, and Hansen (4) questions whether it can be said to occur in this case. He describes the male larvæ as attaching themselves to the female at an early stage, and subsequently undergoing a

<sup>1</sup> Loc. cit., tab. iii.

profound morphological change during which all the larval organs vanish, and the testes together with their efferent ducts are formed. Owing to this transformation Hansen states that the morphological orientation of the adult is uncertain. As, however, the larva is attached by its anterior end, it seems unlikely that the developing male would revolve round in its larval skin, so as to reverse its anterior and posterior ends, hence it is in all probability safe to speak of the openings as occurring at the anterior end of the male.

The loss of the alimentary canal is also interesting, especially as it has practically gone in the female as well, though in this case its place is taken by the absorptive root system. The male seems to be without the means for obtaining nourishment.

#### IV. RELATION OF PARASITE TO HOST.

The most frequent position of the female *Herpyllobius* is on the head of the worm, although it is sometimes attached to a parapodium. Levinsen (9) describes the root system of the parasite as forming a tongue-shaped body lying by the side of the gut of the host, and I have been able to confirm this statement by means of sections. In the worm sectioned the parasite was attached to the head, and the roots actually pierced through the cerebral ganglion of the host (Pl. 22, fig. 8) to reach the alimentary canal, and further on (Pl. 22, fig. 9) could be seen lying by the side of the œsophagus, which had been pushed over to one side by this intrusion. The internal economy of the worm with three parasites on its head must have been extremely deranged, but unfortunately it did not cut well and I was unable to make out the relative positions of the three roots.

The roots obviously have an absorptive function, and the process must be carried on in spite of the protective envelope formed by the worm round it. The lacunar tissue of the root system is in continuation through the stalk with

vacuolated tissue in the external body, as well as with the "gut" spaces, and it is probably by this means that nourishment is carried to the reproductive organs, which occupy practically the whole of the external body.

As to the life-history, there is nothing known. There is probably a free-swimming stage in the female, as in the allied *Lernæopodidæ*, as otherwise one cannot account for the infection of new worms. It is probable that the males become attached to the female at an early stage, as is the case in *Rhizorhina*; having no gut and no apparent means of absorbing nutriment they must be short-lived.

No stages of development were to be found in the eggs.

#### V. COMPARISON WITH ALLIED GENERA.

Hansen, in his work on the *Choniostomatidæ* (4), gives an account of the relationships of *Herpyllobius* and the allied genera. Previously<sup>1</sup> he had established the family *Herpyllobiidæ* containing the seven genera: *Herpyllobius* (Stp. and Ltk.), *Eurysilenium* (M. Sars), *Rhizorhina* (H. J. H.), *Trophoniphila* (McIntosh), *Æstrella* (McIntosh), *Saccopsis* (Lev.), and *Bradophila* (Lev.).

*Trophoniphila bradii* is described by McIntosh<sup>2</sup> as adhering to the bases of the branchiæ of the worm *Trophonia*, while *Æstrella levinseni*<sup>3</sup> is described by the same author from *Ehlersiella*; in both cases, however, an extremely short description is given, so that it is only possible to refer them to this family without any comparison with the other groups. The female of *Rhizorhina* forms a short slender stalk which pierces the skin of the gill of its host (*Ampelisca*); in this stalk are two tubes which, on entering the skin of the host, separate and ramify irregularly through the gill, sometimes entering into the body of the host. In *Herpyllobius* and *Eurysilenium* the stalk pierces the

<sup>1</sup> Hansen, "*Rhizorhina ampelisca*," loc. cit.

<sup>2</sup> McIntosh, "Report on the Annelida Polychæta," 'Report Scient. Results of H.M.S. "Challenger,"' xii, 1885, p. 368, pl. xxxvii, fig. 4.

<sup>3</sup> McIntosh, loc. cit., p. 477, pl. xxxix, fig. 11.

skin of the host and there expands into a collar-like hold-fast, from which there depends an oblong or irregularly-shaped body, which is homologous with the tubes of *Rhizorhina* and has the same function of supplying nourishment to the external body. Levinsen (9) has found that *Saccopsis* and *Bradophila* have stalks, but he has found no expanded body at the end of it in the body of the host; Hansen (4), however, is of the opinion that some tubes must run into the body of the host, otherwise it is difficult to see how the parasites get their food.

A comparison between the two best-known genera of the family *Herpyllobiidae*, *Rhizorhina* and *Herpyllobius*, shows important differences in the stalk of the female, as mentioned above, and also in the male. In both forms the larva fastens itself to the female, after which the tissues of the larva contract, thus forming a sac-like adult, without visible internal organs except the gonads and their efferent ducts. In *Rhizorhina* the male remains entirely inside its larval skin, pushing its generative ducts out through a hole in front of the mouth of the dead case. In *Herpyllobius* the skin of the larva bursts and the male is fastened by the anterior end, the generative ducts proceeding through the split produced by the bursting of the larval skin.

#### VI. AFFINITIES OF GROUP.

Giard and Bonnier (2) regarded the *Herpyllobiidae* as closely resembling the *Choniostomatidae*, for although they considered the females too degenerate to admit of comparison, they believed that the males, especially the larval males, strongly resembled one another both in the mouth parts and in the position of the generative opening. For these reasons they proposed to make a new order, *Sphæronellidae*, containing two subdivisions, the *Herpyllobiinae* and the *Choniostomatinae*.

Hansen (4), however, points out that there are structural differences in the two groups that render such a classification unsatisfactory. The female *Choniostomatid* is less degenerate,

having mandibles, maxillæ and maxillipeds, while the females of the Herpyllobiidae are entirely without appendages. The description of the males is at fault, for the adult male of Herpyllobius and Rhizorhina is limbless and degraded and cannot be said to possess mouth parts, while the male Choniostomatid is highly developed and has well-formed mouth-parts.

It therefore seems best to retain the two as separate families, equivalent to the Chondracanthidae, the Lernæopodidae and others.

It might be of interest at this juncture to make some comparison between the Herpyllobiidae and the Rhizocephala, as each may be considered the most degraded form of the Copepoda and Cirripedia respectively. In both the adult female consists of two parts, one inside and one outside the host, the outside portion containing the reproductive system, whilst the part inside the host forms a root system for absorbing nutriment and conveying it to the external part. Some analogy might also be traced between the chitinous ring of the Rhizocephala and the chitinous hold-fast of Herpyllobius. There is of course a great difference in the method of infection: Sacculina, for instance, obtains entrance into the body of the host as a larva, and undergoes metamorphosis as an endo-parasite, the adult being evaginated at maturity; while Herpyllobius fixes itself to the outside of the host, probably at an early larval stage, and, if it is true that a protective membrane is formed by the worm, remains as an ecto-parasite throughout life.

The Rhizocephala are as a rule hermaphrodite, thus differing from the unisexual Herpyllobiidae, but this is not always the case, for G. Smith<sup>1</sup> has found in the mantle cavity of Duplorbio what appear to be extremely degraded, but still functional males. In the ordinary individual no testes are found, as is also the case in Sylon, but in the latter no male individuals are known.

<sup>1</sup> Smith, G., "Rhizocephala," 'Fauna und Flora des Golfes von Neapel,' Monogr. xxix, 1906.

Another interesting parallel is afforded by the Pedunculate Cirripede *Anelasma squalicola*, which is parasitic on the Selachian genus *Spinax*. G. Smith<sup>1</sup> describes this form as having a root system which arises from the region of fixation and penetrates into the flesh of the shark, probably serving to nourish the parasite, although the gut is not degenerate. The roots closely resemble those of *Sacculina*, having a chitinous investment, below which is a regular epithelium, while the inside is occupied by a lacunar tissue of vacuolated cells. This lacunar tissue is distributed over all parts of the body and may carry nourishment, thus recalling a similar structure in *Herpyllobius*.

Dr. Calman has kindly drawn my attention to the fact that absorptive "roots" are also developed by some parasitic Isopods. Prof. Caullery<sup>2</sup> describes *Wanalia* as forming a tubular prolongation in the region of the mouth which penetrates into its host, *Inachus*, but it is at first separated from the body cavity by a membrane. Two pairs of processes are then formed at the distal end, the mouth opens between them, and the whole system pierces through into the body cavity of the crab. This "proboscis," as Caullery calls it, serves the double purpose of fixation and of absorbing nourishment. A similar organ is found in the genus *Cryptoniscus*.

We thus find a remarkable case of convergent<sup>\*</sup> evolution in the development of absorptive roots in the Copepoda, Cirripedia and Isopoda.

## VII. SYSTEMATIC.

### Generic Characters of *Herpyllobius*.

Female.—Body consists of two parts joined by a stalk. The anterior part (root system), which lies within the host, is soft and elongated, composed of lacunar tissue, and provided

<sup>1</sup> Smith, G., loc. cit.

<sup>2</sup> Caullery, "Récherches sur les Liriopsidæ." 'Mittheilungen aus der Zool. Station zu Neapel,' xviii, 1907-8, p. 583.

with a chitinous fixing organ where it penetrates the tissue of the host. The external posterior part is subglobose, white, and lacking any vestige of limbs. Posteriorly are two chitinous buttresses which support the large egg-sacs, and above are six chitin-surrounded pores, near to which the males are attached. The stalk is joined to the middle of the lower surface of the external part, the interior of which is principally occupied by the reproductive organs.

Male.—Minute and sac-like, consisting only of reproductive organs, anteriorly drawn out into a conical prolongation which is inserted into the female. Forms two large spermatophores with long necks. Most of the body of the adult remains enclosed in the last larval skin, characterised by the following characters: Cephalothorax with two pairs of appendages, antennæ and maxillipeds, the latter with their last joint hooked. Three free thoracic segments, each bearing a pair of biramous swimming legs. Abdomen three-jointed; last joint longest and bearing telson with long bristles.

#### Specific Characters.<sup>1</sup>

*H. arcticus* (Stp. and Ltk.).—Female: Anterior portion 4–5 mm. long,  $\frac{1}{3}$ –1 mm. broad. External portion  $1\frac{1}{2}$ –2 mm. long (without egg-sacs). Stalk very thin, diameter one-eighth of the length of the external portion. Male (Larval skin):  $\frac{1}{8}$ – $\frac{1}{4}$  mm. long. Antennæ three-jointed. Maxillipeds four-jointed. First and second pairs of thoracic legs with external rami two-jointed.

Habitat.—Greenland, on two species of Polynoid—*Harmothoë imbricata* and *Ennoë ørstedii*. Gulf of St. Lawrence, on *Nychia amondseni*.<sup>2</sup> Gulf of Georgia Puget Sound, on *Harmothoë*: Antarctic, on three species of Polynoids.<sup>3</sup>

<sup>1</sup> Levinsen (Θ).

<sup>2</sup> McIntosh, 'Ann. Nat. Hist.,' 4th series xiii, 1874, p. 262.

<sup>3</sup> Gravière, C., "Sur quelques Crustacés parasites annelidicaux provenant de la seconde expédition antarctique française," 'Comptes Rendues,' xlv, 1912, p. 830.



*H. crassirostris* (Sars).—Female: Anterior portion unknown. External portion  $\frac{3}{4}$  mm. long. Stalk very broad, diameter one quarter of the length of the external portion. Male (Larval skin): No antennæ (?). Maxillipeds three-jointed. External ramus of swimming legs one-jointed.

Habitat.—Norway, on *Polynoë imparis*.

*H. affinis* (H. J. H.).<sup>1</sup>—Female somewhat similar to *H. arcticus*, but double the length. Body half as broad as long, subovate. Egg-sacs half as broad as long, fusiform. Length of body 3–4 mm. Male indistinguishable from that of *H. arcticus*.

Habitat.—Karske Hav, Northern Siberia, on *Harmothoë badia*.

There seems no reason for separating the species of *Herpyllobius* of Puget Sound from *H. arcticus*, first described by Steenstrup and Lütken; the size of the female seems the same, and the males appear identical. I therefore propose to call this species *Herpyllobius arcticus*, only recording as a new locality for it the west coast of North America, and it remains as another example of the similarity in the fauna of the North Pacific and Atlantic sides of America.

#### VIII. SUMMARY.

(1) The female *Herpyllobius* is entirely without appendages, and consists of two portions united by a thin stalk.

(2) The rounded posterior or external portion contains the reproductive organs and bears two large egg-sacs. In it are certain spaces with thick epithelium-like walls, which probably represent the gut.

(3) The anterior portion or root system is an elongated body lying within the host, and composed of vacuolated tissue penetrated by a lacunar system.

(4) The lacunar system of the root unites into a main branch, which runs up the stalk and is continuous with the gut spaces in the external portion.

<sup>1</sup> Hansen (3).

(5) The root is, therefore, in all probability an absorbing organ, nourishment being carried to the external portion of the body, which contains the important reproductive organs, by means of the lacunar system.

(6) In one specimen (fig. 5) the body contained numerous eggs lying in the diverticula of the two oviducts, each oviduct terminated in a thick-walled glandular portion. In the posterior region of the animal was a median vesicle communicating on each side with a lateral one; into the duct between the two there opened the glandular oviduct, and from there a short duct led to the exterior.

In this specimen there was no trace of a gut.

(7) The parasite is usually attached to the head of the worm, and in the two specimens cut the root pierced the cerebral ganglion and lay by the side of the œsophagus (figs. 8 and 9).

(8) *Herpyllobius* is one of the seven genera of the family *Herpyllobiidae*.

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### EXPLANATION OF PLATE 22,

Illustrating Kathleen Haddon's paper on “*Herpyllobius arcticus*.”

Fig. 1.—Adult female on *Harmothoë imbricata*, showing the attachment of the parasite to the dorsal surface of the worm's head.

Fig. 2.—Dorsal view of an adult female, showing two males attached posteriorly. On either side of these are the chitinous buttresses supporting the egg-sacs.

Fig. 3.—Side view of a stained specimen after clearing; external portion of the parasite only. The opaque gut lies in the centre sending out radiating channels. Posteriorly is shown a chitinous buttress for the support of an egg-sac.

Fig. 4.—Longitudinal section through an adult female.

Fig. 5.—Side view of the peculiar type, after staining and clearing, showing the arrangement of the genital organs.

Fig. 6.—Transverse section of the above specimen.

Fig. 7.—Transverse section of a normal female, showing the external portion, and the root embedded in tissues of the host.

Fig. 8.—Transverse section of worm's head, showing space (*sp.*) formerly occupied by the roots of the parasite. This pierces the cerebral ganglion of the worm.

Fig. 9.—Transverse section of the same worm farther back, with the root of the parasite (*rt.*) lying beside the œsophagus of the worm.

Fig. 10.—Dorsal view of a male *Herpyllobius* enclosed in its last larval skin.

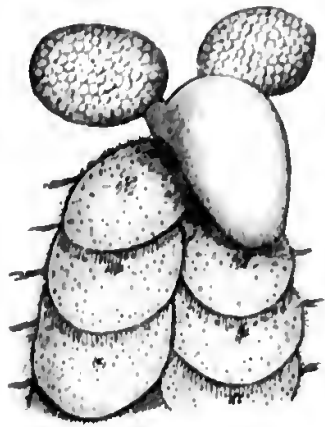
#### LETTERING.

*b.* Buttress supporting egg-sac. *ca.* Canal system of body. *cg.* Cerebral ganglion. *co.* Commissure. *cut.* Cuticle. *cut.*<sup>1</sup> Cuticle of root. *cl.* Elytra of worm. *ep.* Epidermis of worm. *es.* Egg-sac. *fib.* Fibrous tissue. *gl.* Glandular oviduct. *g.o.* Genital opening. *hf.* Hold-fast. *hyp.* Hypodermis. *lac.* Lacunar system of root. *lv.* Lateral vesicle. *m.* Mouth. *mb.* Membrane formed by worm. *mf.* Muscle-fibres. *mt.* Mouth of worm. *mv.* Median vesicle. *od.* Oviduct. *œs.* Œsophagus. *ov.* Ovum. *par.* Parapodium. *rt.* Root of parasite. *s.* Space occupied by parasite. *sp.* Spermatophore. *sp. d.* Duct of spermatophore. *t.* Telson. *v. n. c.* Ventral nerve-cord.

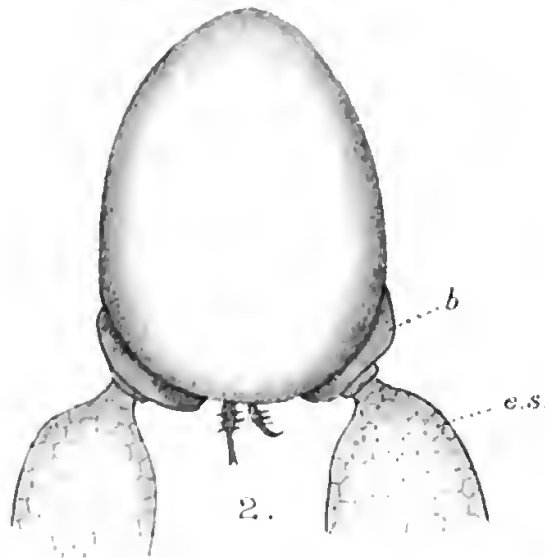








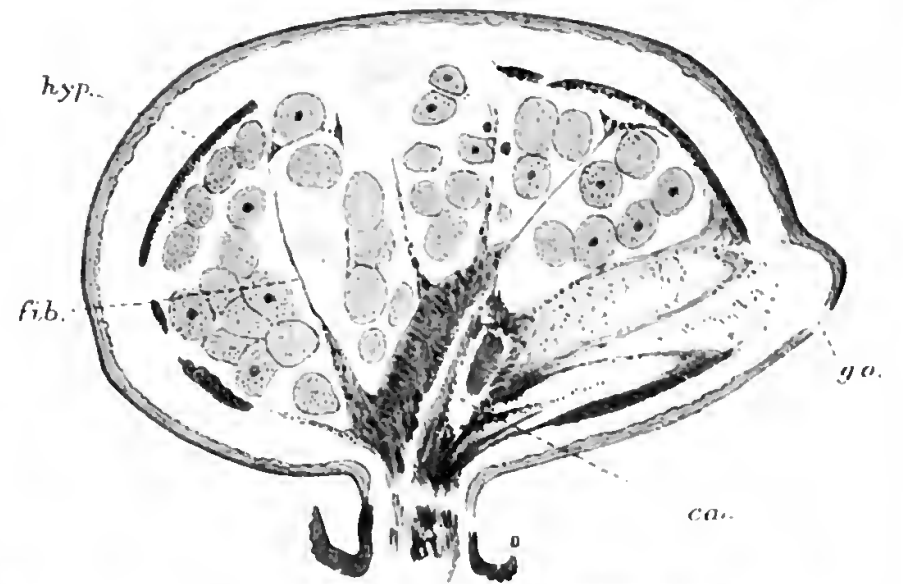
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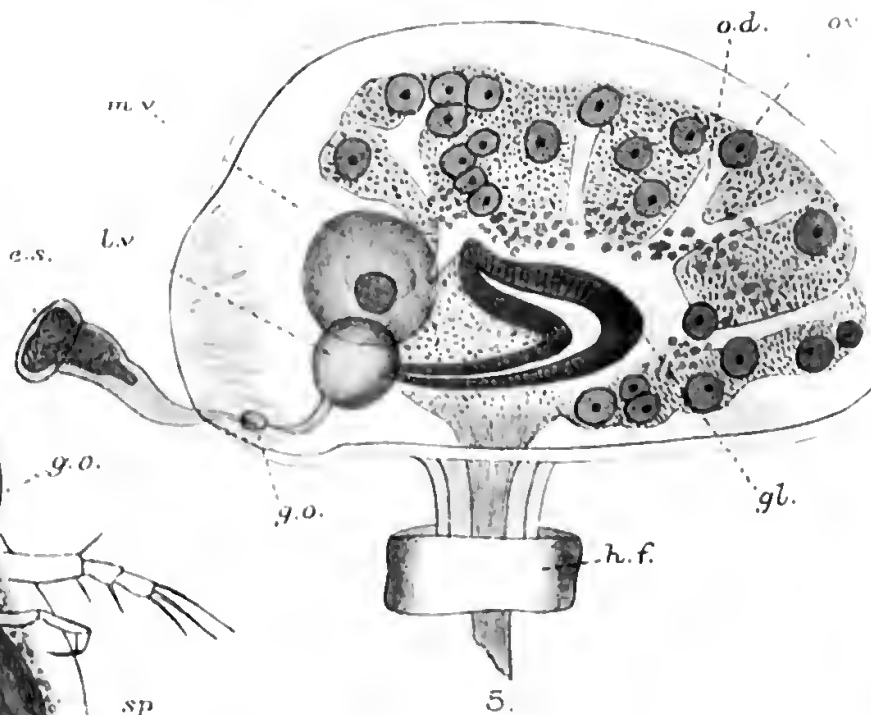
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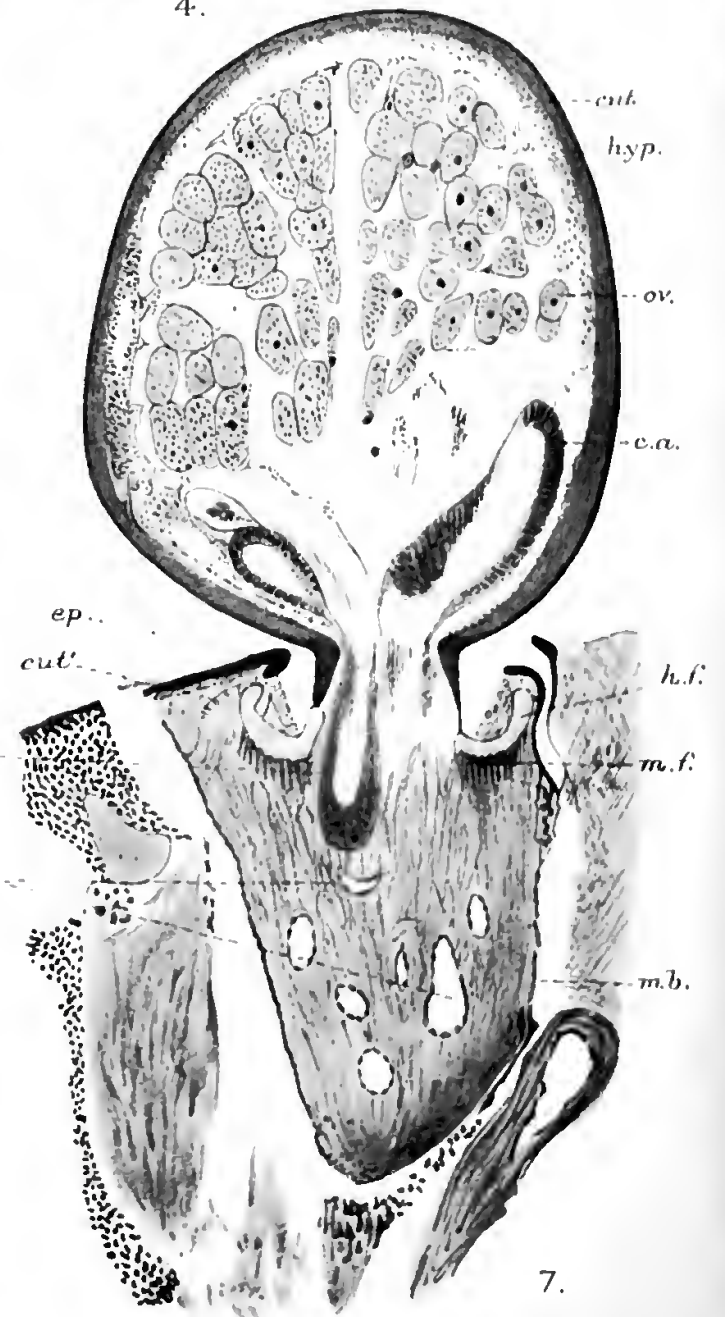
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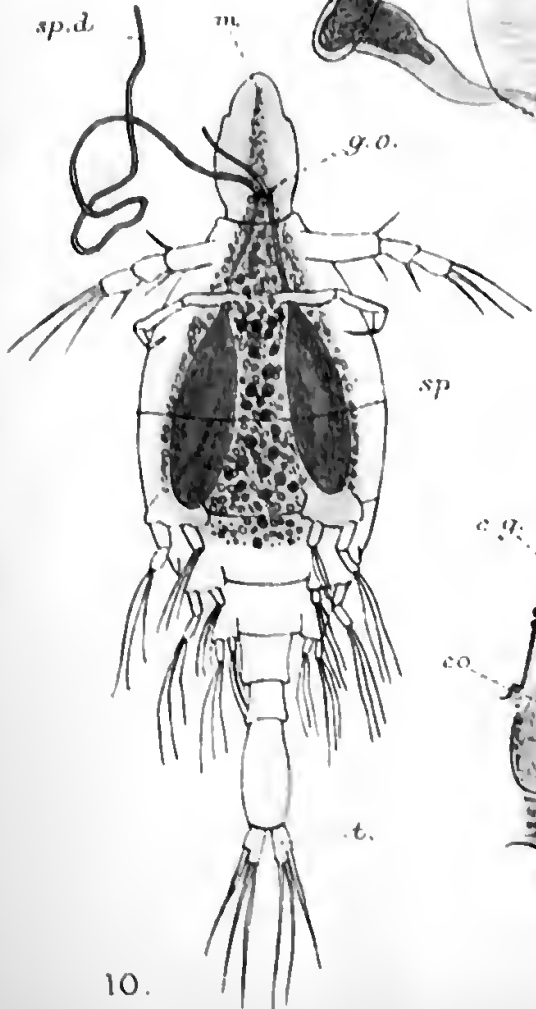
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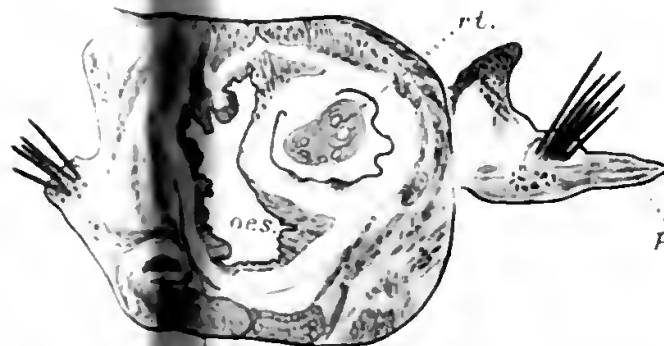
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## Stolon Formation in Certain Species of *Trypanosyllis*.

By

**F. A. Potts, M.A.,**

Fellow of Trinity Hall, Cambridge, and Demonstrator of  
Comparative Anatomy in the University.

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With Plate 23 and 23 bis and 8 Text-figures.

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### INTRODUCTION.

IN 1896 a single specimen of a Syllid Polychæt was discovered by the Columbia University Expedition to Puget Sound, in which the reproductive individuals or stolons were produced from the ventral surface of the last two segments to the number of fifty or more, arranged in a compact rosette instead of such a linear chain as occurs in *Autolytus* or *Myrianida*. This was described by H. P. Johnson (1) as *Trypanosyllis gemmipara*. For another unique example exhibiting a similar phenomenon, collected by Harold Heath in California, he instituted the species *Trypanosyllis ingens* (3). Since then two other species have been discovered which reproduce in the same manner—*T. misakiensis*, by Izuka, in Japan (4), and *T. crosslandi*, by Cyril Crossland, at Zanzibar, which is here fully described for the first time.

Though the phenomenon has thus been shown to exist in species of the genus *Trypanosyllis* from widely different parts of the world, it has never been found possible to obtain material in sufficient quantity for a thorough study of the phenomenon. In fact, only a single stolon-forming individual

had been obtained of each of the first three species and three of *T. crosslandi* when I summarised our knowledge of this method of reproduction in 1910 (5). During the summer of the next year, 1911, in the course of a visit to British Columbia, I was able to search for *T. gemmipara*, and, though my success was not so ample as I had hoped, I am able to give a fuller account of this specialised form of budding than has been published before. I should like here to record my indebtedness to Dr. C. F. Newcombe, of Victoria, whose knowledge of the marine zoology of various localities in British Columbia was most useful to me in my quest for *T. gemmipara*.

#### TRYPANOSYLLIS GEMMIPARA JOHNSON.

The account by Johnson, both of the systematic characters and of the formation of stolons in this species is full and correct. On a few points I find it possible to add materially, and it seems necessary to give specific confirmation of others.

#### OCCURRENCE.

The single specimen obtained by the Columbia University Zoological Expedition in 1896 probably came from the neighbourhood of Port Townsend, but unfortunately no data accompanied it when it was handed over to Johnson for description. From Johnson's third paper (3, p. 306, footnote) it appears that he re-discovered the species in small numbers on the Californian coast, but he gives no further information as to its occurrence.

In the summer of 1911 I obtained this species from three separate localities in the N.W. Pacific. Though widely distributed it is only to be obtained during the lowest spring tides, and is even then comparatively rare in the restricted habitat it frequents. I give details as to localities below.

(1) Cape Beale, on the west coast of Vancouver Island, at the mouth of Barkley Sound. A single incomplete specimen,

collected in the littoral zone during the lowest spring tide at the end of May.

(2) Off Brown's Island, Friday Harbour, San Juan Island, State of Washington, U.S.A. Another single specimen, which came out of material dredged at a moderate depth.

(3) In the neighbourhood of Victoria, Vancouver Island. Five individuals (one reproducing) were collected at the time of the very low spring tide on June 27th, at Macaulay's Point, a locality made classical by Lord's description in 'The Naturalist in British Columbia' (Vol. II., p. 12).

They were obtained under masses of Halichondroid sponges, only uncovered at the lowest point of the tide, and appear to exist in rounded tunnels in the sponge, or between the sponge and the rock, which they have themselves eaten out. There was no exit to the exterior or any indication that the worm usually leaves the shelter of the sponge.

Three other specimens (one the posterior end of a reproducing individual) were found during the low tides of the two previous days on the shore near Beacon Hill Park. Here they existed amongst sponges and other encrusting organisms hidden under masses of kelp roots (*Costaria*).

The animals are striking and even beautiful in appearance. The largest was 94 mm. in length and 3 mm. in breadth, but there is a striking variation in size. The body is generally light brown or lemon-yellow, with the diverticula of the intestine showing through as a darker brown. The long moniliform dorsal cirri are generally white or pale pink, often touched with crimson or purple pigment, developed in some specimens over the whole cirrus, and it is their curling form and touches of colour which give a distinctive appearance to the species. In the individuals possessing stolons these are lemon-yellow in colour like the stock.

#### PRELIMINARY DESCRIPTION OF THE MATERIAL.

Various data with regard to the development of the worms collected are given below in tabular form. Numbers are

given to the individuals to which it will afterwards be convenient to refer:

	Length	Width.	Approximate number of segments.	Condition of posterior region.
Victoria :				
I	19 mm.	2 mm.	Head fragment.	—
II	22 „	2 „	110 segments + tail of about 30 segments.	Tail only .6 mm. in length.
III	41 „	2 „	220 segments.	Tail long, graduating insensibly into stock.
IV	45 „	3 „	220 segments.	Ditto.
V	55 „	3.5 „	195 segments + tail of about 40.	Tail 2 mm. long without caudal cirri.
VI	72 „	4 „	172 segments + tail of about 100.	Tail 14 mm. long.
VII	85 „	2.5 „	Incomplete.	—
VIII	94 „	3 „	356 segments.	Cluster of young stolons at tail.
IX	—	—	Tail fragment.	Cluster of more advanced stolons.
Friday Harbour :				
X	66 mm.	3 mm.	125 segments + tail of 90 or more.	—

The specimen originally procured by the Columbia University Expedition had the following dimensions:—

Length.	Width.	Approximate number of segments.	Condition of posterior region.
68 mm.	3 mm.	300 or more segments.	Clusters of more than 50 stolons.

It is seen that a complete worm varies greatly in length from 22 to 94 mm., and contains 140 to 356 segments. In most of the specimens, as the table shows, there is present a tail of smaller and less developed segments. In II this is extremely rudimentary, though even then containing thirty segments or more. The figure here given of it (Pl. 23, fig. 1) shows that these segments are less developed as traced backwards to the posterior end where proliferation takes place and new segments are added. In the development of the para-

podia it will be seen that the dorsal cirrus appears first as a club-shaped structure, which is articulated only in the oldest segment of all, while the neuropodium, with its setæ, develop soon afterward. The ventral cirrus, however, did not seem to be present at this stage. It may also be noted that in this early condition the anal cirri are not developed, and a contrast is thus established with the production of new stolons in *Autolytus* and *Myrianida*, where the anal segment is the first to be formed (cf. Potts, 5, p. 39, ff.).

In other specimens the tail is met with in a condition of increasing length and development until it contains more than 100 segments, the oldest of which approximate in width and depth to the segments of the stock (compare especially III and IV). In the reproducing individual (VIII) alone, however, are the segments from head to tail of approximately equal development.

It may be suggested that the possession of such a tail as is described above denotes a period of recovery between two periods of reproduction. In one individual (III) there are three successive constrictions or scars at equal distances apart, which seem to indicate pauses in the growth of the worm, such as might correspond to recurring sexual periods. From the fact that in both the two complete reproducing specimens the number of segments should be about 350, it by no means follows that the younger worms forming the great majority of the population should not be capable of reproduction at an earlier period. It seems more likely that worms, with the sedentary habits characteristic of *Trypanosyllis gemmipara*, live to an age of several years, growing to a greater length each year, and probably producing an annual crop of stolons. After the liberation of these mature reproductive buds (which does not involve the sacrifice of an actual portion of the stock, as in the case of so many Syllids) rapid regeneration takes place, and after new segments to the number of 100 or more have been formed, these attain gradually the development of the older segments of the stock, when reproduction will again take place. Hence

we may expect to find reproducing individuals differing widely in length.

The principal interest lies in the two specimens which are producing stolons (VIII and IX). In the individual from Port Townsend, described by Johnson, the stolons were about fifty in number, arising from the right-hand border of the last segments of the stock. Thirteen of them had attained an advanced stage of development (with twenty to twenty-eight segments—2.5 mm. in length). "To the right at the base of the cluster is a group containing about twenty-five very young buds as yet showing no segmentation, but each with the two distal processes which are the anal cirri." "The buds are arranged in rows, partly transverse and partly longitudinal as regards the axis of the stock." "In a graduated series, proceeding from the left side of the cluster of youngest buds, are more advanced buds, the largest approaching the mature condition."

It is evident that Johnson's individual was characterised by very asymmetrical development of the proliferating patch. No. VIII of my collection (Pl. 23, fig. 2) resembles his in all essential particulars, but it will be noticed that the zone of proliferation occupies an entirely median position, and the stolons are at first arranged in perfectly transverse rows, the members of which are all equally developed, even to sharing a lateral twist, which directs the tails of all the stolons in one row to the right and to the left in the next.

As the stolons increase in size and breadth the outer members of the row are pushed more and more to the side, and also anteriorly, because of the presence of the rows behind, so that the row becomes a crescent, and the proliferating region is partly surrounded by stolons in advanced development. Irregularities must, however, frequently occur owing to inequalities of growth. The asymmetry of Johnson's specimen, it appears to me, is due, firstly, to the lateral appearance of the patch of proliferation, and, secondly, to distortion, due to unequal growth of the members of the different rows, which have had more room to grow on the left of the stock,

and so have developed there more quickly. Johnson's drawing (3, fig. 8) shows clearly the conversion of the earliest formed transverse rows into semicircles surrounding the area of proliferation.

In IX (Pl. 23, figs. 4, 5) the area of proliferation is again situated centrally. The first-formed stolons are, however, older than those of VIII, and many of them have been removed or have fallen off, giving an appearance of irregularity.

#### THE GENITAL APPENDAGE OF THE STOCK.

I wish here to emphasise one point in Johnson's description which is, I believe, a peculiar characteristic of *Trypanosyllis gemmipara* compared with the other forms with related reproduction. It will be worth while to quote at length the passage bearing on this point:

"Occupying the most dorsal position among the advanced buds is the attenuated and rapidly diminishing caudal extremity of the stock. It is much like the buds in general aspect, and further resembles them in containing sperm-cells which are absent in all portions of the stock in front of the proliferating region. It differs from the buds in possessing (1) a heavily ciliated continuation of the alimentary canal (fig. 7, *al. c.*); (2) an anus; (3) and differs in lacking cephalisation. In every respect, except that it contains sperm-cells, it resembles a regenerated posterior extremity. It possesses twenty-four segments, including the pygidium. The proliferating region is, therefore, this number of segments in front of the posterior extremity. As fig. 7 shows, the attenuated posterior end has almost as much the appearance of being an appendage to the budding zone as any of the mature buds."

In Pl. 23, fig. 2, a dorsal view of VIII is given, showing this "caudal extremity," or genital appendage of the stock, as I propose to call it, turned backwards by the growth of the stolons ventral to it. Neither the stolons nor the "tail"



itself are so far developed as in Johnson's form. The latter, instead of possessing caudal cirri, ends perfectly bluntly, so that apparently the number of segments is not yet quite complete, and those formed are much narrower than those of the stock. It thus appears much more clearly than in Johnson's specimen that this appendage is of recent growth. It is right, I think, to regard proliferation of stolons as always taking place terminally, the regeneration of an appendage to the stock being a contemporaneous phenomenon. In both cases the determining cause of formation is the onset of sexual maturity, for gonads are developed in each segment of the newly formed extremity of the stock as well as in the stolons. A particularly interesting development of the phenomenon is exhibited in IX, where the dorsalmost stolon (Pl. 23, fig. 6) was detached soon after preservation. It was 3.5 mm. in length, contained thirty-four segments, and had a distinctly developed head, with eyes. Evidence of the existence of an alimentary canal appeared in a black swelling in the middle line of the stolon, and the existence of an opening, apparently the anus, just ventral to the caudal cirri. On cutting a series of transverse sections the gut was seen to be complete from mouth to anus, and functional, as witnessed by contained food masses, largely consisting of sponge spicules. There can be no doubt that this stolon, the only one of the large number developed which possessed the least vestige of an alimentary canal, is to be homologised with the genital appendage of the stock as observed by Johnson and myself. It may even be suggested that this structure normally develops a head, and becomes detached as a stolon at a late period of development, but it is at least equally likely to be an individual variation in this particular case. But I think it is fair to regard this terminal stolon or appendage as a survival from a time when the ancestor of *Trypanosyllis gemmipara* reproduced by means of a single terminal stolon, especially if detachment proves to take place usually.

TRYPANOSYLLIS CROSSLANDI SP.N.<sup>1</sup>

This was obtained in the harbour of Wasin, Zanzibar. The following account of its occurrence and appearance is compiled from Mr. Crossland's notes. In colour it is a bright orange-red; the four eyes are brown. The stolons are of the same colour as the stock. In life, Mr. Crossland remarks, their tails keep waving in the water, but whether this is because of muscular movements of the stolons themselves preparatory to detachment from the stock, or is merely due to the currents in the water, he does not make clear. The animal appears to live in a red sponge.

## DIAGNOSIS.

Length, 22 to 25 mm. Breadth, 3 mm. (including setæ). Number of segments, 125 to 135.

Trypanosyllis of small size and number of segments. Colouration as above. Prostomium (Pl. 23, fig. 7) small, not distinctly bilobed, but tripartite; eyes raised on slight conical elevations, which coalesce in case of front pair. Two pairs of eyes, arranged almost in a square, but posterior rather wider apart and more minute. Median and lateral tentacles about the same length, with fifteen or so annulations. Dorsal cirri slender, alternately long and short, with unusually long unjointed base, succeeded by thirty to forty annulations in the case of the longer, and twenty to twenty-two in the shorter, the whole sometimes longer by one third than the breadth of the body. Parapodia (Pl. 23 bis, fig. 19) acuminate, apex occupied by three short acicula, and below these eight to twelve stout compound setæ. In these the terminal piece, with incurved apex, has a smooth internal margin; shaft below provided with short processes, giving a pectinate appearance (Pl. 23 bis, fig. 18).

Pharynx lined by a very thin chitinous tubular lining; the

<sup>1</sup> Named after my friend Mr. Cyril Crossland, Clare College. Cambridge, and now Biologist to the Red Sea Pearl Fisheries, who collected this and many other interesting forms at Zanzibar in 1900-1.

aperture into pharynx sheath surrounded by eight fleshy rounded lips. The trepan of teeth marking the anterior end of the chitinous lining is much reduced, the teeth being only represented by crenulations of the chitin. No accessory tooth behind the trepan.

Reproduction by collateral budding of stolons (for details see below).

*T. crosslandi* is a well-marked species. It differs from most of the members of the genus (except *T. zebra*) in its small size, from all others except *T. ingens* in the structure of the ventral setæ, the terminal piece of which has a smooth internal margin, without accessory tooth or pectinate edge (e.g. figs. 17 and 18 in Pl. 23 bis), and from every species, as far as I know, in the great reduction of the cuticle and armature of the pharynx. From the other three species which reproduce by collateral budding *T. crosslandi* is easily distinguished by these and other points.

#### REPRODUCTION IN *TRYPANOSYLLIS* *CROSSLANDI*.

The chief difference from *T. gemmipara* is found in the absence of a genital appendage to the stock.

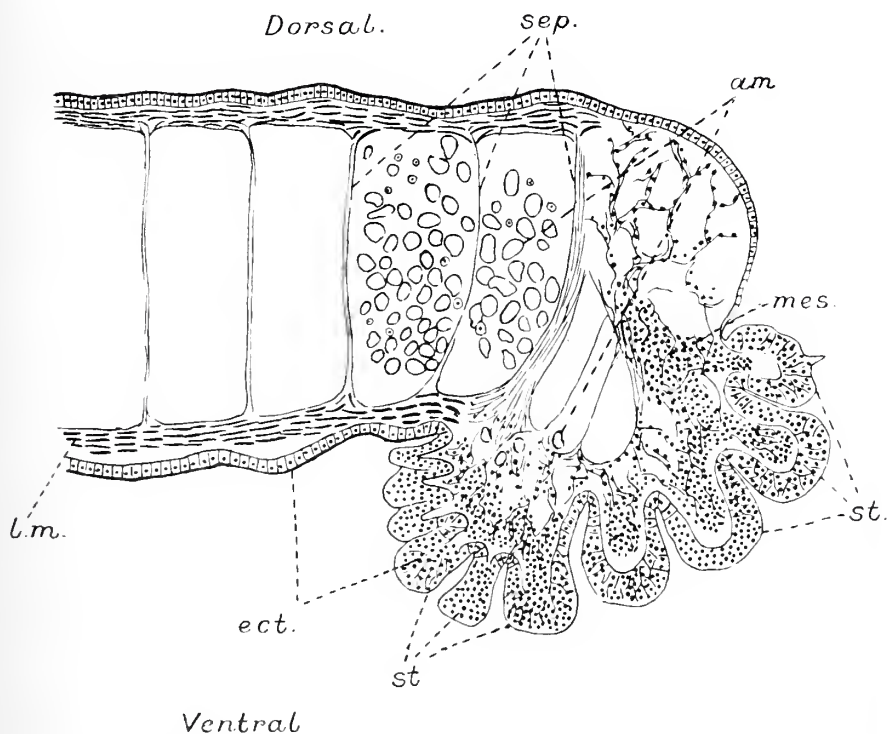
Three individuals only were preserved, but this small series represents as many different periods of stolon-formation, namely, the beginning of proliferation, full maturity and cessation of activity. The following brief descriptions may be given of the three forms:

(1) A male individual with a small oval patch of proliferating tissue on the ventral surface of the last two segments. A vertical longitudinal section (Text-fig. 1) shows that the stolons are all in the first stages of development, that none as yet are segmented, though as many as nine rows have been formed, packed closely together.

(2) Another male individual, with about 100 stolons of all sizes (Pl. 23, figs. 8 and 9). The largest stolons were ready for detachment and amply provided with swimming setæ. The area of proliferation is displaced to the right side, and the regular transverse rows of stolons described above in

*T. gemmipara* are not displayed here. New stolons are still being formed, but the effect of the great amount of growth which has taken place in the posterior rows has been to draw out the end of the stock into a sort of pedicle, which has been turned back over the dorsum, so that the anus

TEXT-FIG. 1.



Vertical longitudinal section through posterior end of stock to left of middle line (*T. crosslandi*, 1), showing a newly formed proliferating cushion with several rows of stolons established, all as yet unsegmented. *am*, Leucocytes, free in coelom and invading mesoblast. *ect*, Ectoderm. *mes*, Mesoderm. *l.m.*, Longitudinal muscles of stock. *sep*, Septa in stock. *st*, Rudimentary stolons.  $\times 120$ .

points anteriorly. This gives off secondary processes, to which the now fully formed stolons are attached, each by a distinct stalk.

(3) A female individual with four stolons only (Pl. 23, fig. 10). These are attached to a narrow terminal pedicle which is the dwindled representative of the terminal segment. Three of the stolons are of large size, and it is evident that this individual is in the last stage of reproduction, when proliferation has ceased and the major portion of the stolons are detached. Dorsal to all is a minute prominence which bears the anus (not shown in figure but hidden behind stolons). Even at this late stage there is no beginning of regeneration of fresh segments.

This individual consists of 136 segments altogether. After the ninety-seventh there is a marked diminution in the width of the segments, and it is likely that the succeeding segments represent growth after an earlier period of reproduction.

#### COMPARISON OF THE STOLONS OF THE TWO SPECIES.

The stolons of *T. crosslandi* differ in one or two characters from those of *T. gemmipara*. In the first place, in correlation with the smaller size of the stock, they have fewer segments and are shorter than those of the latter species. The number of segments is never more than eighteen, and the longest stolon measured 2.5 mm. In *T. gemmipara* the number of segments lay between twenty and twenty-eight, and the average length was 2.5 mm.

In the structure of the head, the stolons of *T. crosslandi* show themselves more advanced than those of *T. gemmipara*. Both forms possess two pairs of eyes and a brain, but the former also (Pl. 23 bis, fig. 20) have a pair of minute lateral tentacles. Palps are apparently absent but dorsally in the angles between the head and the succeeding segment are a single pair of well-developed tentacular cirri of greater length than the dorsal cirri of the body segments. This comparatively advanced condition of the head ("tête dicère") is attained before the separation, and is common to stolons of different sizes. Possibly subsequent development of other head appendages may take place.

In *T. gemmipara* Johnson says that "the head is merely

the eye- and brain-bearing first segment which shows its primitive character by also carrying parapodia equipped with dorsal cirri, setæ and acicula." I have confirmed this in the examination of a large number of the stolons of the Victoria specimens. In the possessions of the typical "tête acère" *T. gemmipara* resembles *T. krohnii* (= *T. zebra*), and *T. crosslandi* is thus the only member of the genus which produces so advanced a type of stolonial head.

A third point of difference presents itself in the development of the caudal cirri. In *T. gemmipara* they are the earliest and most prominent features, and are much longer, thicker, and consist of more annuli than the dorsal cirri of the posterior segments, in stolons of all stages of development. In *T. crosslandi*, on the other hand, they are so small as to be hardly noticeable. They are equalled in size by the dorsal cirri of the stolon, although these themselves are less developed than in *T. gemmipara*.

It should be noticed that the setæ of the stolons of both species resemble each other in type. At first appearance the terminal piece, triangular in shape, appears to have a smooth internal margin, but closer examination shows a minute tooth just under the apex. This seems to suggest that *T. crosslandi* is derived from a species (like *T. gemmipara*) with such a seta in the adult.

#### THE INTERNAL CHANGES ACCOMPANYING STOLON-FORMATION.

The earliest stage of the phenomenon is shown by (1) of *T. crosslandi*, in which several rows of stolons have been marked out but none of the stolons have become segmented. This was examined by means of a series of vertical longitudinal sections, and typical members of these are drawn in Text-figs. 1 and 2. It is here shown that proliferation has given rise to a cushion of undifferentiated tissue standing out from the ventral surface of the stock. It is formed by ectoderm and mesoderm alone, and the former is indented by furrows which mark off the incipient stolons.

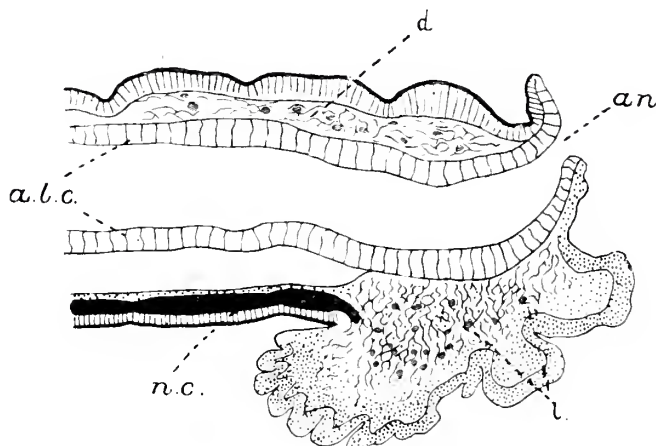


On tracing the tissues from the stock into the area of proliferation the following changes may be seen to take place:

(1) The cuticle becomes very much reduced in thickness.

(2) The ectoderm increases in places to nearly twice its original thickness by continued division of its cells. But along certain lines little nuclear division takes place and the thickness remains that of the ectoderm of the stock. In fact it may be said that within the area of proliferation there exist as many centres of proliferation as there are stolons to

TEXT-FIG. 2.



As in Text-fig. 1, but representing a median section. The nerve-cord is shown, stopping short of the proliferating cushion. The centre of the latter is seen to consist mainly of a meshwork of fibres, between which have penetrated large leucocytes. *n.c.* Nerve-cord. *al.c.* Intestine. *an.* Anus. *d.* Dorsal extension of mesoblastic tissue. Other letters as in Text-fig. 1.  $\times 70$ .

be produced. But anteriorly fresh centres are being constantly formed from a proliferating lip.

(3) The proliferating area is formed by the last two segments of the stock. In the last segment of the stock the body-cavity is largely occupied by a proliferation of mesoblastic tissue (Pl. 23 bis, fig. 11), which penetrates into and forms the basis of the cushion formed by the proliferation of the ectoderm. It consists of a meshwork of connective tissue,



the spaces between which are occupied by (1) large oval nuclei with small scattered chromatin granules (*mes. n.*), (2) much smaller nuclei with a large intensely staining nucleolus (*l.*).

The body-cavity of the two penultimate segments is almost filled by leucocytes (Text-fig. 1; Pl. 23 bis, fig. 12). These are of two types: either with an abundant investment of cytoplasm, and laden with food material (*l.*), or with a mere superficial film of cytoplasm (*l'*). Both may be spherical or show amoeboid processes, and both possess small nuclei, with large intensely staining nucleolus. It is the existence of large numbers of nuclei of this type in the mesoblast, as noted above, that shows immigration of leucocytes to have taken place to a great extent. The larger type of leucocyte has undoubtedly, I think, a nutritive function, and may be observed in the meshes of the connective tissues, with the cytoplasm entire or partially disintegrated. But the smaller kind of leucocyte is too small to carry nourishment, and I am inclined to think that these wandering cells undergo a nuclear change, and become the actively dividing cells of the mesoblast. In the latter stages, which have been examined by sections, no trace remains of this collection of leucocytes in the posterior segments—that is, they have all been absorbed to add to the numbers of the proliferating cells or to nourish them for their task.

VIII of *T. gemmipara* exhibits a further stage in development. As compared with (1), it will be seen that the cushion of proliferation has increased in size, and projects beyond so as to overlap two segments in front of its point of origin. Posteriorly the growth of the stolons has been so marked as to press back the genital appendage from the position it should occupy as the prolongation of the stock, until it stands nearly at right angles to its former position. The number of rows of stolons is increased to about eleven, and it is seen that fresh rows are in process of being cut off from a proliferating lip.

The preservation of this specimen is more favourable than

that of any others in my possession to an exact study of the histology. It will be best to first consider the conditions at the proliferating lip (*pr. l.*) where fresh stolons are being formed (Pl. 23 bis, fig. 13). The epiblast of the cushion is at first of a normal type, narrow columnar cells with small nuclei containing scattered granules of faintly staining chromatin. There then occurs a distinct furrow, which separates this region from the triangular proliferating lip. Here the cells alter their character, the nuclei becoming larger and rich in darkly staining chromatin, generally massed in two (or sometimes more) nucleolar masses. Several cells, markedly larger than the rest, are to be met with in which there is a single large nucleolus. These changes are observed in the epithelium of the upper surface only; the cells of the lower surface remain short and small, with faintly staining nuclei passing again at the trough of the furrow into columnar cells.

The mesoblast of the interior consists of very numerous cells, embedded in a fibrous matrix. The cells are frequently spindle-shaped. The chromatin of the nucleus consists of a single deeply staining nucleolus and other smaller granules, and mitoses are relatively common, while they are rare in the epiblast. As the mesoblast enters the proliferating lip, and is traced dorsally, changes occur similar to those noted in the epiblast—the cells increase in size and the nucleolus tends to increase in bulk and staining qualities. But though undergoing similar modification there is nearly always a distinct line between ectoderm and mesoderm, which is never crossed by migrating cells.

Next to the proliferating lip is the youngest stolon (*st'*), pear-shaped, with a narrow stalk, and as yet without any beginnings of segmentation. The histological characters are like those described in the preceding paragraphs, and it is suggested that young stolons of this character are occasionally cut off from the proliferating lip. But even at this early stage there is a considerable difference between the two surfaces of the young stolon. The upper surface (*vent.*), where the epiblast is greatly thickened, becomes the ventral surface of

the stolon. It is from this surface, of course, that the parapodia, with their setæ and parapodial glands, will afterward develop, and the ventral ectoderm of the adult stolon is of much greater thickness and importance than the dorsal. These facts account for the early difference between the surfaces. At first the ventral epithelium consists of a single layer of very deep cells, but in the succeeding stolons it becomes several cells in depth.

The mesoblast of the interior of the stolon becomes applied to the epiblast of the ventral surface, though there is always a definite line between the two. But between the mesoblast and the dorsal epiblast there is from the beginning a space which becomes more marked as development proceeds. The next change which is noticed in the stolons is the segmentation of the mesoblast (Pl. 23 bis, fig. 14). From the ventral mass of undifferentiated material there grow out laterally and dorsally thin septa with a fibrous basis invested by globular mesoblast cells which become the peritoneal epithelium. This segmentation establishes itself very speedily; while the mesoblast of one stolon is still undifferentiated that of the next oldest is almost completely segmented. Only at the anterior end, in the pedicle, and in the freely projecting posterior end does the mesoblast remain entirely unsegmented.

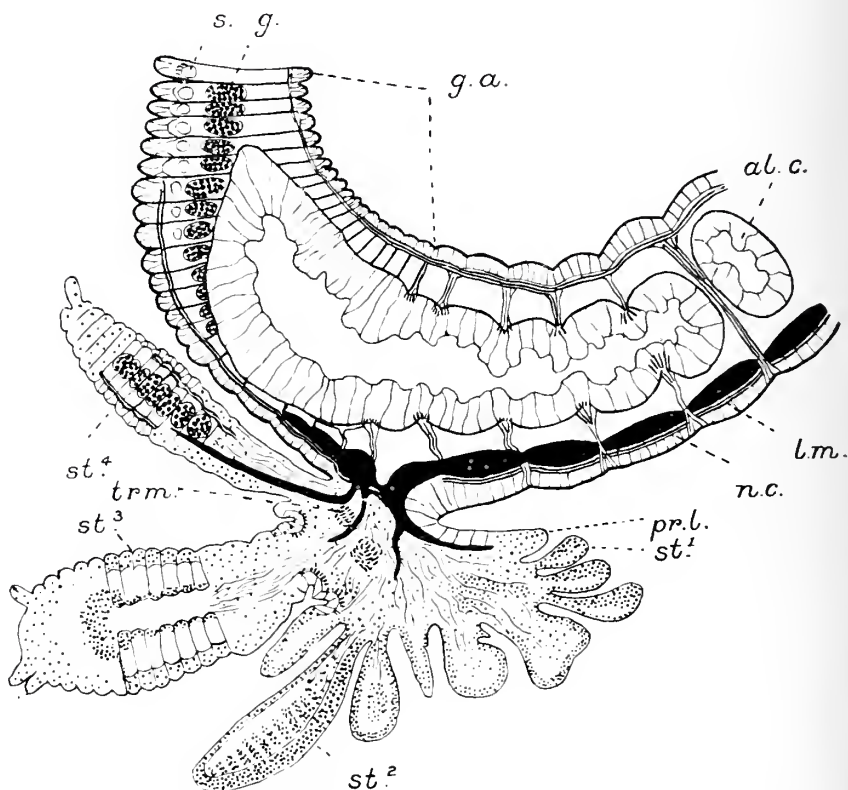
But while the segmentation of the mesoblast is thus shown in the formation of the septa and later the growth of the gonads, there remains at first a central mass of mesoblast, which gradually dwindles, however, until there is merely a central unsegmented longitudinal wisp of tissue—which occupies a dorsal position in the stolon—consisting of fibres with a few scattered nuclei (Pl. 23 bis, fig. 15 *mes. r.*).

It is not for some time afterwards that the ectoderm begins to share in the segmentation, the parapodia being then really formed by the pressure of the proliferating mesoblast beneath. But at this stage the setæ form and the darkly staining gland cells appear in the epithelium (Pl. 23 bis, fig. 15).

In the development of the stolon, although the greater part

of the tissues and organs have to be differentiated from the proliferated epiblast and mesoblast, the two most important systems, the nervous and muscular, are at first formed as direct

TEXT-FIG. 3.

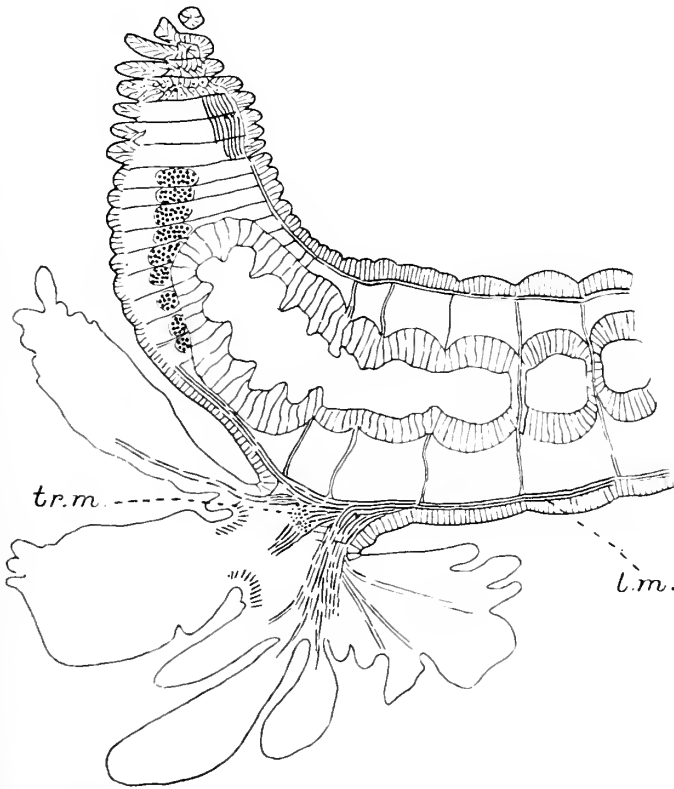


Median longitudinal section through posterior end of budding stock (*T. gemmipara*, VIII), showing branching of nervous system represented by thick black lines. *gen.a.* Genital appendage of stock. *pr.l.* Proliferating lip of cushion. *st¹*. Youngest stolon. *st²*. Stolon with mesoblast just beginning to segment. *st³*. Stolon with median region segmented cut in horizontal section. *st⁴*. Stolon with differentiated gonads. *tr.m.* Transverse muscle-bands in cushion. *s.* Aciculum. *g.* Gonad.  $\times 70$ .

continuations of the original systems of the stock. It is at this stage of development that they are first seen in the stolon.

In Text-fig. 3 the ventral nerve-cord is shown entering the region of proliferation, where it becomes thinner and loses the directness of its course, at the same time giving off a large and important nerve which runs into the proliferating

TEXT-FIG. 4.



Longitudinal section from same series as Text-fig. 3, showing the branching of the muscular system. The muscles alone are shown in the proliferating cushion and stolons as parallel black lines. Lettering as above.  $\times 70$ .

lip. Branches from this approach the developing stolon and we see one nerve about to penetrate the pedicle of a stolon, and this occurs as soon as the segmentation of the mesoblast begins. In the last segment of the stock the interrupted

nerve-cord forms a prominent ganglion, and at this point it gives off two distinct nerves, one of which can be traced along its whole course into the dorsalmost stolon. From this ganglion a continuation of the ventral nerve-cord penetrates the genital appendage of the stock, but after the first few segments it becomes almost indistinguishable.

The continuity of the muscles of stock and stolons is even more easily traced. Johnson states that budding of stolons takes place "from a mass of undifferentiated tissue, traversed by muscle-fibres which are continuous with the longitudinal muscle-bands of the buds." Text-fig. 4 represents another longitudinal vertical section through VIII of the *Victoria* individuals. On reaching the region of proliferation the longitudinal muscles of the stock are seen to give off a thick bundle of fibres which turns off almost at right angles, traverses the mesoblast of the cushion, splitting up into smaller bundles which approach the developing stolons. The dorsalmost stolons are supplied by muscle-bundles coming off separately from a continuation of the ventral longitudinal musculature which enters the genital appendage of the stock.

It will be noticed that in nearly all the sections a great many of the muscle-fibres, instead of running in the vertical plane of the section, are gathered into one or two transverse bundles (*tr. m.*), and so are parallel to the direction of the rows of stolons. Transversely arranged fibres also occupy the folds between stolons just under the ectoderm, and it may be noticed, too, that a couple of muscle bundles often enter each stolon, each apparently forming the entire musculature of one side.

We find on examination of *T. crosslandi*, (1) represented in Text-fig. 2, that this representative of an earlier stage of proliferation shows a cushion consisting of connective-tissue fibres only. The ventral nerve-cord of the stock ends bluntly when just penetrating the new tissue. Muscle-fibres seem to be entirely absent. We are thus able to say that the ingrowth of the nervous and muscular systems does not begin till the



cushion of proliferation has been well established and the growth of the stolons themselves has begun.

There is no sign in my material of the migration of nerve-cells into the stolon from the stock. It is therefore quite clear, as Mr. Goodrich has pointed out to me, that these invading nerve-fibres form a kind of provisional nervous system and must die on the detachment of the stolon and consequent separation from the parent cells. But, as is seen later (p. 432), as the ectoderm of the stolon differentiates a regular cellular investment of the nerve-cord appears, and the cells composing it probably develop nerve-fibres which replace functionally the original invaders.

#### THE FURTHER CHANGES IN THE STOLON.

In Pl. 23 bis, fig. 15, is seen a transverse section through a stolon of *T. gemmipara* (VIII), in which the generative glands are developing. In fig. 14, a part of the very same section, they were not to be distinguished from the ventral mass of mesoblast between the septa, but now, in all the segments already formed in the stolon, a pair of oval glands (probably testes) is at present lying almost free in the body cavity, but still united by a ventral yoke of mesoblast, containing cells of the same character as the glands. The generative cells have large nuclei with conspicuous nucleoli, and mitoses are fairly frequent. The cells of the peritoneal epithelium are rounded and very numerous. Dorsally there is only a single layer of cells, but ventrally they are massed together, several deep. The fibrous basis of the mesoblast (*mes. r.*), while it formed the undifferentiated core of the stolon, still remains as a dorsal mass, from which most of the nuclei have migrated, and which is situated between the ectoderm and the mesodermal epithelium.

The musculature is now arranged in four longitudinal bands (*l. m.*). There are as yet no differentiated muscles in the parapodia, which are here developed as merely ectodermal projections, consisting merely of neuropodium and dorsal cirrus. Short acicula have already appeared.



In the ectoderm the nerve-cord is now a double structure, and opposed to it is a crowded mass of nuclei mainly developed in the basal ends of the ectodermal cell impinging on it.

The stolons of this stage consist of a number of segments (fifteen or sixteen), but new segments continue to be added, both proximally and distally. A definite head has not been formed, although the tail segment is early established, and so there is as yet no sign of eyes or of a brain.

In Pl. 23 bis, fig. 16, we have a transverse section through a stolon of *T. crosslandi* (2), the tissues of which have attained to more marked development than any of the stolons of *T. gemmipara*. This has accompanied the ripening of the generative cells. The spermatocytes have escaped from the gonads, and lie freely in the body-cavity. The growth of these has accelerated the shrinkage of the fibrous core of the mesoblast, which now appears as a triangular split between the two layers of peritoneal epithelium. The other great difference has been brought about by the development of the parapodia and muscular system. The notopodia, with bundles of swimming setæ, have appeared. The neuropodia project outward considerably, and their acicula penetrate the cavity of the segment almost to the centre. The four main longitudinal bands of muscle-fibres remain, but there are in addition, in each segment, dorsal transverse bands which supply the swimming setæ, and ventral transverse bands which work the neuropodial setæ, beside the muscles which move the acicula. A close examination of these secondary muscle growths shows their derivation of the four original muscle bands.

The nerve-cord now shows a regular cellular investment, and so while the fibrous core is derived by ingrowth from the stock, the investing layer, which probably forms the permanent nervous system of the stolon, is formed from the ectodermal epithelium.

There occurs also, for the first time, a pair of conspicuous tubular glands in each segment, consisting of a single layer

of vacuolated cells. From their position I think there is no doubt that they are nephridia, but it is exceedingly difficult to make out their internal opening. They occur both in *T. gemmipara* and *T. crosslandi*.

These bodies are figured and described by Johnson, but he chooses to regard them as parapodial glands, identical with those structures in other Syllids. I think that he is without doubt wrong, for the parapodial glands are not hollow structures such as we find here. He says, moreover, that "the glands are not only relatively but absolutely larger in the caudal segments (i. e. in the genital appendage) and in the buds than in the stock," and this fact, which is confirmed in my material, points to a direct connection with the occurrence of the sexual products in these parts. We know that the nephridia function as generative ducts in Syllids and increase in size at reproductive maturity, but there is no reason for regarding the parapodial glands, whose office is the secretion of mucus, as in any way correlated with the generative organs.

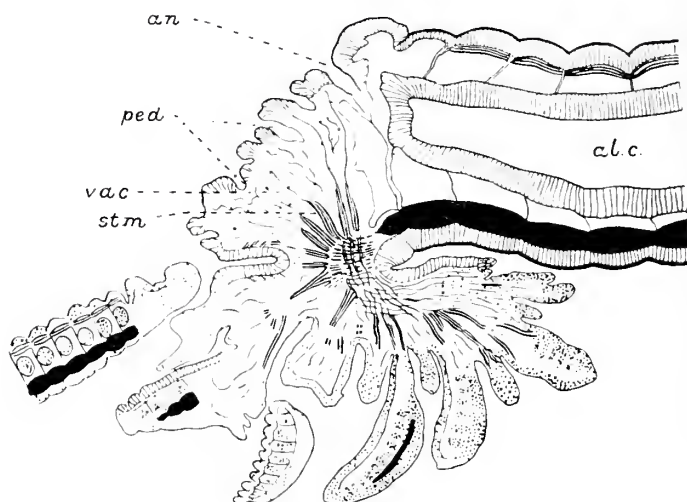
In *T. crosslandi* (3) only a few mature female stolons remained attached to the stock, and one of these which was cut in transverse section showed the whole of the coelom to be crammed with large-sized eggs. The body-wall was reduced to a thin skin, the parapodial muscles and the setæ but slightly developed, and the notopodium hardly perceptible. The stolon was, in fact, reduced to a bag of eggs, and it is difficult to imagine the mature female bud as capable of locomotion.

A word or two may be added to emphasise the late formation of the head. It is probably the last segment to be established. The brain, it is stated by Johnson, develops independently of the nerve-cord by the modification of a mass of ectoderm cells, and he states that the eyes are innervated, not from the brain, but from the nerve-cord. Undoubtedly the brain is only differentiated when the full number of segments have been formed in the stolon, and the other head structures, such as eyes, are being developed.

## THE PROLIFERATING CUSHION IN LATER STAGES.

Text-fig. 5, a longitudinal section through the posterior extremity of *T. gemmipara* (IX), shows the changes in the proliferating cushion which accompany development of the stolons. It will be noticed that the size of the cushion has increased greatly with the number of the stolons. The genital appendage, which appears in fig. 4 as if it would restrict the

TEXT-FIG. 5.



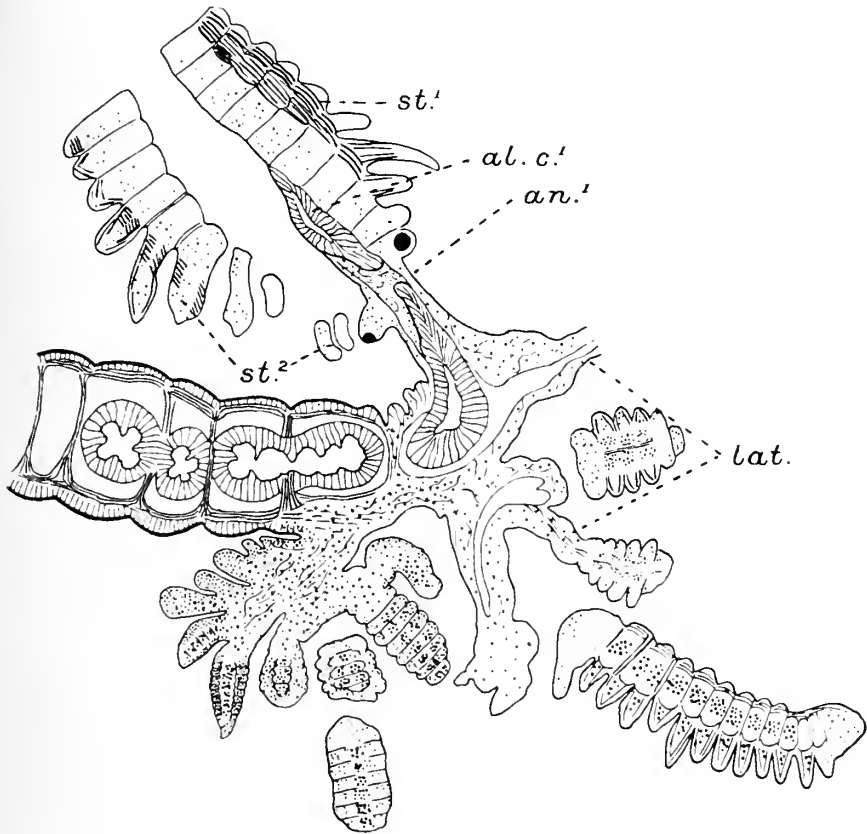
Longitudinal section through posterior end of budding stock (*T. gemmipara*, IX) after detachment of dorsal stolons. *an*, Anus. *ped*, Position from which a stolon has been detached. *st.m.*, Bands of longitudinal muscles supplying stolons. *vac*, Vacuolated mesoblast in the exhausted portion of the proliferating cushion.  $\times 50$ .

growth of the cushion, is not present in this section. It has been pointed out above that it has probably developed as a stolon, remaining attached only by a pedicle, and was detached soon after preservation. The oldest stolons, pushed dorsalward by the growth of new rows behind them, have all matured, and were also inadvertently detached in examining the animal. But there remains in the cushion of proliferation the bundles of muscle-fibres running from stock to stolon, and

the points (*ped.*) at which the ectodermal epithelium is interrupted indicate the situations from which they were detached.

But together with the extension of the proliferating cushion

TEXT-FIG. 6.



Longitudinal section through posterior end of stock (*T. crosslandi*, 2) showing an advanced stage of stolon formation. *st*<sup>1</sup>, *st*<sup>2</sup>. The two first formed stolons. *lat.* Lateral processes of last segment-bearing stolons. *al. c*<sup>1</sup>. Diverticulum of alimentary canal in dorsal-most stolon. *an*<sup>1</sup>. Position of anus. Other letters as above.  $\times 70$ .

there has been a change in its histological character. At the stage represented by figs. 3 and 4 the whole of the cushion is closely packed with mesoblastic cells. Here this character is

still preserved in the proliferating lip and its neighbourhood, but in the parts opposite more fully developed stolons the interior of the cushion is occupied by scanty and vacuolated tissue, consisting only of the basis of fibres, connective-tissue and muscular, which can be noticed in the younger parts. But the cellular elements are well-nigh absent, and it must be supposed that they migrated into the stolons while they were attaining their great development. The ectodermal epithelium, too, is much vacuolated and contains sparsely scattered nuclei.

Text-fig. 6, representing *T. crosslandi* (2), shows a still more advanced stage. That part of the cushion where proliferation is still going on and the cells of the mesoblast still fill the interior is reduced to small proportions. The rest of the cushion, extending far dorsalward, has thinned out considerably, being absorbed in long processes which support small groups of stolons. It is quite evident that when proliferation has finally ceased and the stolons have all broken away, the cushion disappears and the body-wall is reduced to its normal proportions.

#### THE CŒLOMIC CAVITY DURING PROLIFERATION.

During the early stages of proliferation the cœlom does not extend into the cushion, although the leucocytes probably invade the tissue in great numbers and play a most important part. In fact the cavity of the cœlom is encroached upon and almost obliterated by the proliferation of mesoblast, which is not confined to the ventral cushion but also goes on dorsally (see *T. crosslandi*, Text-fig. 2).

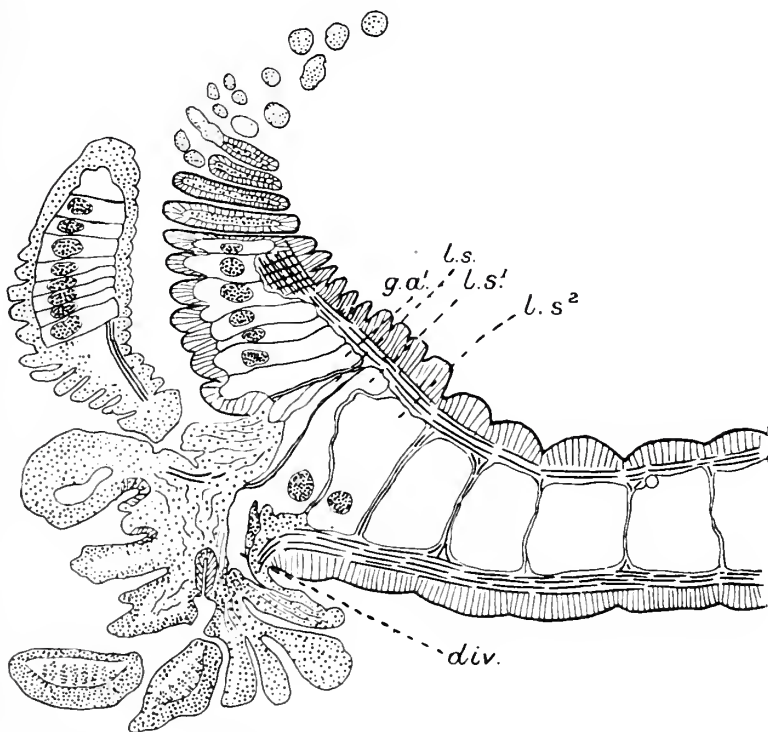
In *T. gemmipara*, at a later stage (Text-fig. 7), a diverticulum of the cœloomic cavity does invade the cushion in the neighbourhood of the proliferating lip. The cœloomic epithelium here appears to be specially thickened, but I do not know if the cells which compose it migrate into and reinforce the mesoblast of the cushion.

In *T. crosslandi* at a still later stage (Text-fig. 6) the cœlom is prolonged into the lateral outgrowths of the cushion from which the stolons depend.

NUMBER OF SEGMENTS CONCERNED IN THE REGION OF  
PROLIFERATION.

In two of the examples sectioned it was possible to distinguish clearly that the proliferating cushion occupied a part of

TEXT-FIG. 7.

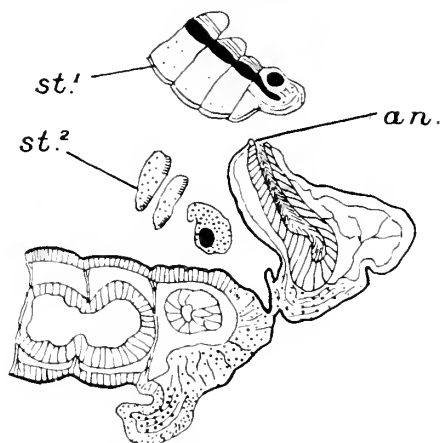


Longitudinal section as in Text-figs. 3 and 4, but more laterally, showing distribution of gonads in stock and appendage, and extension of coelom into proliferating cushion. *div.* Diverticula of coelom. *l.s.*, *l.s¹*, *l.s²*. Last three segments of the stock. *g.a¹*. First segment of the genital appendage. Other letters as above.  $\times 70$ .

the two segments of the stock (*T. gemmipara*, Text-figs. 4, 6; *T. crosslandi*, Text-fig. 1). In Text-fig. 6 of *T. crosslandi* it is probable likewise that two segments have been concerned. In the long-drawn-out terminal part we pro-

bably have a single segment. In the remaining individual of *T. gemmipara* (Text-fig. 5) it is impossible to trace the limits of the proliferating zone. It seems, then, usual for the cushion to be formed from the ventral surface of two segments. But variability is so great in the transverse situation of the cushion that it probably occurs too in its longitudinal extension. It seems, however, quite certain that the proliferation takes place at the terminal segments, and not, as Johnson supposed, at a segment some distance from the

TEXT-FIG. 8.



Part of section adjacent to that represented in Text-fig. 7, showing anus of stock.

end, for the genital appendage of *T. gemmipara* is also a recent growth from these same terminal segments.

The occurrence of gonads in the genital appendage and the stock itself.—The salient facts in the structure of the genital appendage of *T. gemmipara* have been correctly stated by Johnson and are quoted above. In my specimen (VIII) it may, however, be mentioned, a pair of gonads is developed in every segment, and also in two preceding segments of the stock itself (Text-fig. 7). The occurrence of sexual products in the last segments of the stock is



by no means uncommon in the Syllids; but it is interesting to find it in so specialised a case as this.

#### REPRODUCTION IN TRYPANOSYLLIS INGENS.

In the single specimen of *T. ingens* (3, pp. 296-302) the stolons had nearly all been detached during preservation. It is stated that their place of origin is not terminal, but about twenty somites from the posterior end. No generative products were found in the stock, but Johnson thinks that they are probably developed in "the parental somites posterior to the budding zone." It seems likely, from these data, that *T. ingens* conforms to the type of reproduction found in *T. gemmipara*, a genital appendage being formed about the same time as the stolons.

The most noteworthy point which appears in Johnson's description is the higher development of the stolons compared with those of *T. gemmipara* and *crosslandi*. This is expressed in the occurrence of (1) a "minute median tubular structure," which Johnson interpreted as a rudiment of the alimentary canal, and (2) a dorsal and ventral blood-vessel (but no lateral vessels). These are important differences, but in Johnson's drawing it is not made clear that the "rudimentary gut" is lined by a definite epithelium, and unless this is so there is no real difference between this structure and the mesoblastic residual strand in the stolons of *T. gemmipara* and *T. crosslandi*. The presence of blood-vessels, too, needs confirmation in a better-preserved specimen.

#### GENERAL CONSIDERATIONS.

In the papers of Johnson the phenomenon here described is referred to as collateral budding, and this nomenclature has been followed by Izuka and myself. I wish, however, to state my grounds for maintaining this term, which are possibly different from those which led Johnson to adopt it. In the specimen examined by him the area of proliferation has a markedly lateral position, occurring, indeed, on a

level with the parapodia of one side. This position caused Johnson to describe the stolons as lateral outgrowths of the stock (3, p. 311), and to apply the name of "collateral budding" to the phenomenon. My observations lead me to consider the area of proliferation as arising typically in a median position, the frequent displacements from the middle line being variations therefrom. The correct description of the origin of the stolons from the stock is given in the term ventro-terminal budding, for the stolons are always ventral outgrowths from the stock and from its last segments. My reason, however, for retaining the name collateral budding as applying likewise to the phenomenon is found in the disposition of the stolons in rows, so that they lie side by side, and not end to end. In fact they arise collaterally with each other and not with the stock.

Unfortunately, in my paper already cited, I referred to the phenomena in *Trypanosyllis* and in *Syllis ramosa* under the same name (5, p. 22), though recognising the wide difference in the methods of budding in the two genera. It is with the view of correcting this error that I take the opportunity of tabulating and defining the three methods by which stolons are budded amongst the Syllids. I propose to substitute the adjective "lateral" for "collateral" in referring to *Syllis ramosa*.

Linear budding (terminal). Stolons produced at the end of the stock, and arranged end to end in chains. *Autolytus* and *Myrianida*.

Lateral budding. Stolons produced singly as lateral outgrowths from the stock. *Syllis ramosa*.

Collateral budding (ventro-terminal). Stolons produced from a ventro-terminal proliferating cushion on the stock, and arranged side by side in rows. *Trypanosyllis gemmipara*.

It is interesting to note a point in common to *Trypanosyllis gemmipara* and *crosslandi*. Both, like *Syllis ramosa*, live and feed on sponges, and as far as I can tell only leave their retired habitat under exceptional circum-

stances. This no doubt goes some way to explain the "large size and heavy form" which Johnson noticed in *T. gemmipara*, suggesting "that they are somewhat sluggish in their habits," but it would no doubt be erroneous to suppose that the aberrant method of reproduction was due to the habitat, for *Haplosyllis*, with its simple method of reproduction, is likewise a sponge-dweller. We can, indeed, say that the habit of stolon reproduction in the Syllids generally is partly due to the sheltered and isolated localities they inhabit, but to attribute any extreme form of this to a particular habitat would be rash.

For the present the problem of collateral budding in *T. gemmipara* and the other species must remain almost isolated. But two circumstances help us to form some idea of its origin. The first is the formation of the genital appendage and its occasional separation as a stolon, which points to the derivation of *T. gemmipara* from a species like *T. krohnii*, in which a single stolon is formed at a time, from the posterior segments of the stock. The second is the occurrence in this latter species of a kind of ventral budding from the extremity of the stock to form a second stolon. A full description of this remarkable phenomenon (see 5, pp. 20-22) is out of place here, but it seems to me that an exaggeration of the tendency might well result in the production of a number of ventral stolons, while the retarded formation of the first stolon would give rise to the organ described here as the "genital appendage."

#### REMARKS ON *T. MISAKIENSIS* IZUKA (4).

Only a single individual of this species has been obtained and I am not certain that a sufficient case has been made out for separating it from *T. gemmipara*. Its length, breadth and number of segments fall easily within the limits recorded for *T. gemmipara*. In two other important points—the presence of bidentate ventral setæ and the character of the head and eyes—the two species agree exactly. Notes on the coloration were not preserved and the account of the struc-

ture of the pharynx is not clear. The stolons of *T. misakiensis* appear to resemble those of *T. gemmipara* in the absence of tentacles, but it cannot be decided from the figure or description whether the head segment actually carries parapodia as in the latter species. The number of segments in the stolon is nineteen, agreeing with *T. crosslandi* (eighteen), and not *T. gemmipara* (thirty), but it is possible that the full number of segments had not been attained.

The most curious point about the stolons is the fact stated by Izuka that "the alimentary canal is the direct continuation of that of the mother individual; it is slender, and terminates at the anus of the anal segment." The presence of the alimentary canal is thus lightly dismissed. We are not told whether this fact was confirmed in a number of stolons and by means of sections, but the example drawn in Izuka's fig. 4 has the flat appearance characteristic of the gutless stolons and shows no sign of the anus. Until a more exact description is given there remain two possibilities with regard to *T. misakiensis*:

(1) That the stolons all possess an alimentary canal, thus representing a more primitive stage than *T. gemmipara* or *crosslandi*, or even that *T. ingens*, which is described as possessing a rudimentary alimentary canal (3, p. 299, fig. 6).

(2) That the dorsalmost stolon (or genital appendage as in *T. gemmipara*) was alone examined and found to possess an alimentary canal, a condition assumed to occur in the others, too. I must confess to a strong suspicion that *T. misakiensis* will be found to exhibit phenomena similar to *T. gemmipara*. But at the same time conditions are so variable among this group of Syllids that it would not be very surprising to find that the stolons still possessed a rudimentary gut.

#### SUMMARY.

In several species of *Trypanosyllis* the stolons are produced from a cushion of proliferating tissue at the posterior

end of the stock in successive transverse rows of seven or eight, the number produced by a single individual being between one and two hundred. New rows are established at the anterior end pushing those already formed backwards so that the oldest stolons are most posterior. Ectoderm and mesoderm alone take part in the formation of the stolons so that they are without an alimentary canal. Two slightly varying types of the phenomenon have been distinguished in the two species here studied.

*T. gemmipara*, ?*T. misakiensis*,  
and *T. ingens*.

*T. crosslandi*.

The phenomenon of stolon formation here is associated with the rapid addition to the stock of a tail of forty to fifty segments. This develops generative glands, like the stolons, but unlike them, contains a direct prolongation of the alimentary canal of the stock. It seems likely that under certain conditions this tail may actually develop eyes anteriorly and separate off as an individual stolon, differing from the others only in its complete alimentary canal.

The stolons produced have a head of the "Tetraglene" type with eyes but without tentacles or palps. The caudal cirri are well developed and the number of segments varies up to thirty.

Stolon formation is not accompanied by regeneration of a posterior tail (which takes place, however, as soon as stolon formation is over). But there is occasionally at least an incomplete ingrowth from the ventral lip of the anus into the dorsal-most stolon, which thus forms a structure in some degree homologous with the tail of *T. gemmipara*.

The stolons have a head with a pair of lateral tentacles. The caudal cirri are but slightly developed, and the number of segments is never more than eighteen.

The following divisions correspond roughly to the stages observed in the growth of stolons. In no example was it possible to examine the early formation of the proliferating cushion.

(1) The aggregation of leucocytes in the posterior segments, which invade the mesoblast of the proliferating cushion.

(2) The appearance of centres of proliferation in the epiblast, which cause the formation of stolons. The mesoblast

advances and fills the hollow processes formed by the ectoblast. It lies at first in close association with the ventral surface of the stolon.

(3) The mesoblast of the stolon proliferates and segments. The first appearance of segmentation is the formation of septa.

(4) The invasion of the stolon by two bundles of muscle-fibres in direct continuation with those of the stock, and a single ventral nerve-cord, also an outgrowth of the corresponding structure in the stock.

(5) The segmentation of the epiblast of the stolon and the formation of the structures derived from it (setae, etc.). The gonads are formed as lateral outgrowths from the central mesoblastic mass.

With the growth of the stolons the proliferating cushion is gradually absorbed.

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#### EXPLANATION OF PLATES 23 AND 23 BIS,

Illustrating Mr. F. A. Potts’ paper on “Stolon Formation in Certain Species of *Trypanosyllis*.”

#### ABBREVIATIONS.

*ac.* Acicula. *a.* Anus. *al. c.* Alimentary canal. *dors.* Dorsal surface of stolon. *epi.* Epiblast. *d. c.* Dorsal cirrus. *g. a.* Genital appendage. *gon.* Gonads. *l. m.* Longitudinal muscles. *l.* Leucocytes. *l’.* Leuco-

cytes with abundant cytoplasm. *mes.* Mesoblast. *mes'*. Unsegmented mesoblast of stolon. *mes. n.* Nuclei of mesoblast. *mes. r.* Fibrous residue of mesoblast of stolon. *ped.* Pedicle of stolon. *n. c.* Nerve-cord. *n. s.* Notopodial setæ. *n. m.* Notopodial muscles. *pr. l.* Lip of proliferating cushion where formation of new stolons takes place. *sep.* Embryonic septum in stolon. *sp. c.* Spermatocytes. *st'*. First-formed stolon. *t. c.* Tentacular cirrus. *v. c.* Ventral cirrus.

## PLATE 23.

Fig. 1.—Ventral surface of *Trypanosyllis gemmipara* (II), showing regenerating tail.  $\times 50$ .

Fig. 2.—Ventral surface of posterior end of reproducing individual (VIII), showing youngest rows of stolons.  $\times 24$ .

Fig. 3.—Dorsal surface of same, showing older stolons and genital appendage of stock.  $\times 20$ .

Fig. 4.—Ventral surface of posterior end of another reproducing individual (IX).  $\times 12$ .

Fig. 5.—Dorsal surface of same.  $\times 12$ .

Fig. 6.—Dorsal view of stolon (detached genital appendage) from IX.

Fig. 7.—Dorsal view of head of *Trypanosyllis crosslandi*.

Fig. 8.—Ventral surface of posterior end of reproducing male (ii).

Fig. 9.—Dorsal surface of same, showing mature stolons attached to attenuated terminal segment.

Fig. 10.—Ventral surface of posterior end of reproducing female (iii). Most of the stolons had detached themselves and proliferation ceased.

## PLATE 23 BIS.

Fig. 11.—Longitudinal section showing a small part of the proliferating cushion in *Trypanosyllis crosslandi* (I) bounded by alimentary canal, showing nuclei of connective tissue and invading leucocytes.  $\times 1320$ .

Fig. 12.—Longitudinal section from the same series as fig. 11, showing boundary of free space of coelom and mesoblast of proliferating cushion. Leucocytes of two kinds free and invading mesoblast.  $\times 1320$ .

Fig. 13.—Section through proliferating lip and youngest stolon in *T. gemmipara* (VIII).  $\times 550$ .

Fig. 14.—Longitudinal section through young stolon in same series as Fig. 13, showing segmentation of mesoblast and the first entrance of nerve-fibres.  $\times 250$ .



Fig. 15.—Transverse section through older stolon in same series, showing formation of the gonads from a central mass of mesoblast.  $\times 330$ .

Fig. 16.—Transverse section through a mature male stolon of *T. crosslandi*.  $\times 330$ .

Fig. 17.—Typical ventral seta of *T. gemmipara*.  $\times 90$ .

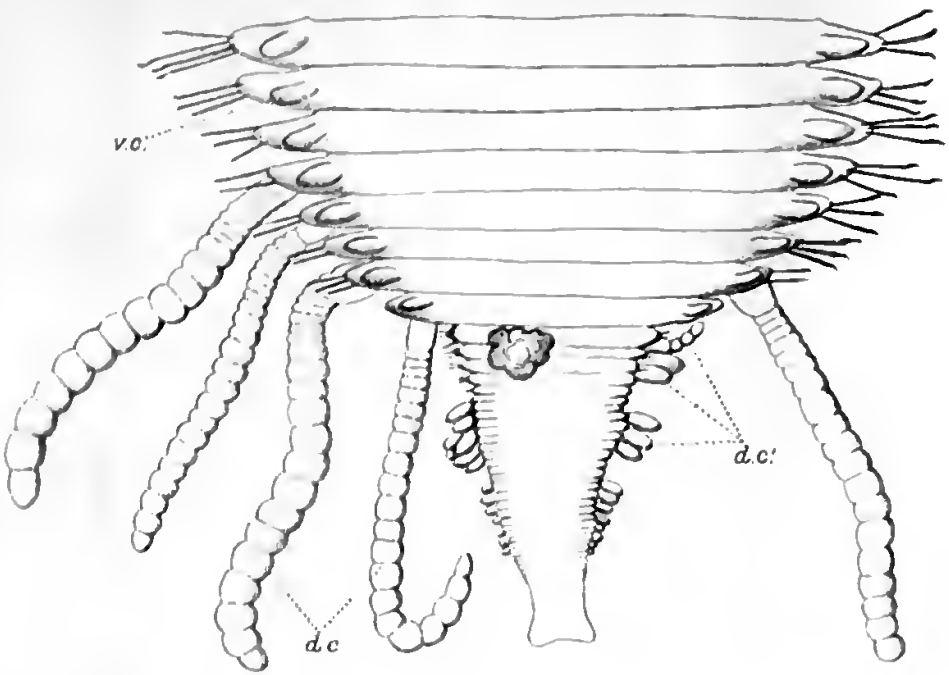
Fig. 18.—Typical ventral seta of *T. crosslandi* (twenty-fifth segment).  $\times 90$ .

Fig. 19.—Parapodium of *T. crosslandi*.  $\times 25$ .

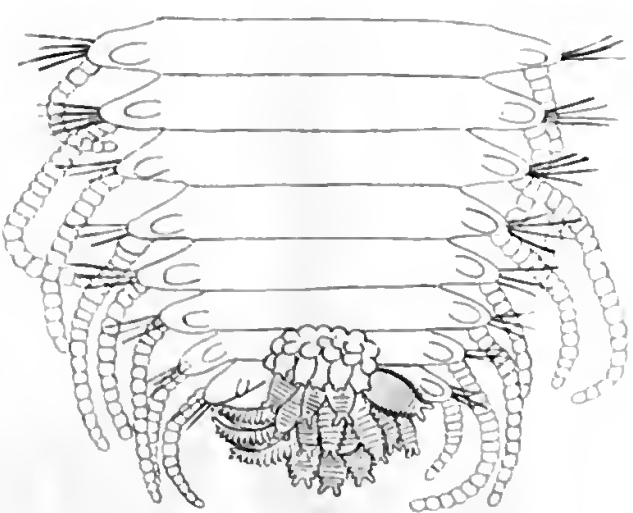
Fig. 20.—Head of stolon of *T. crosslandi*.



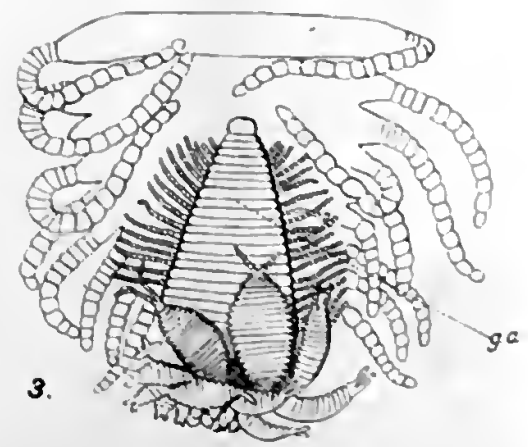




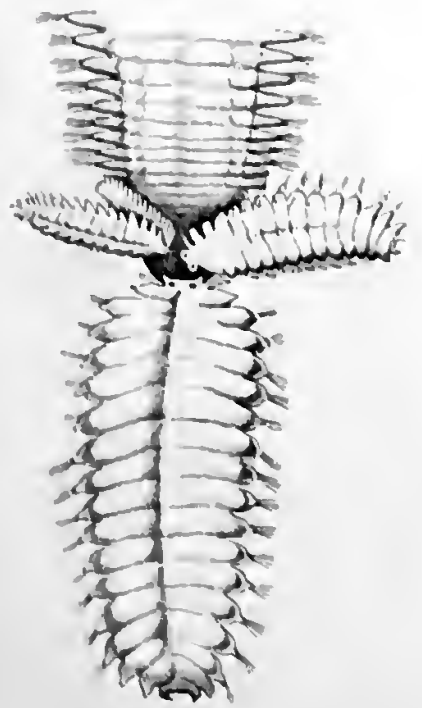
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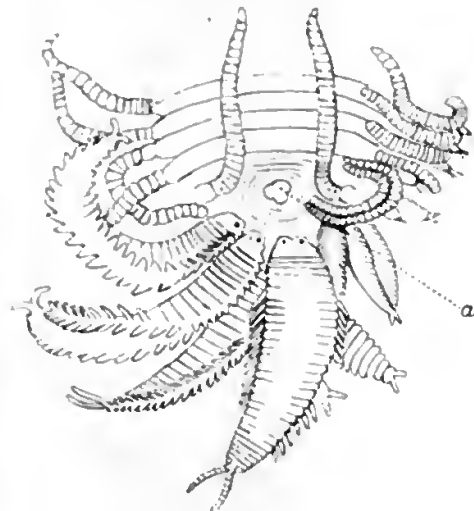
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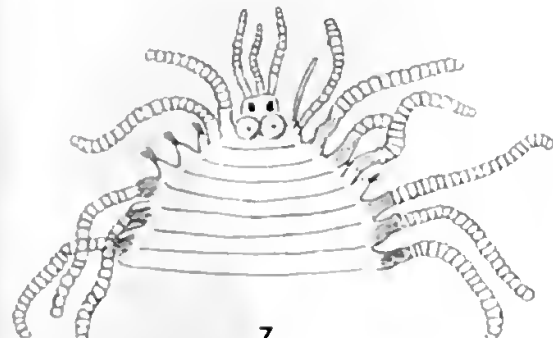
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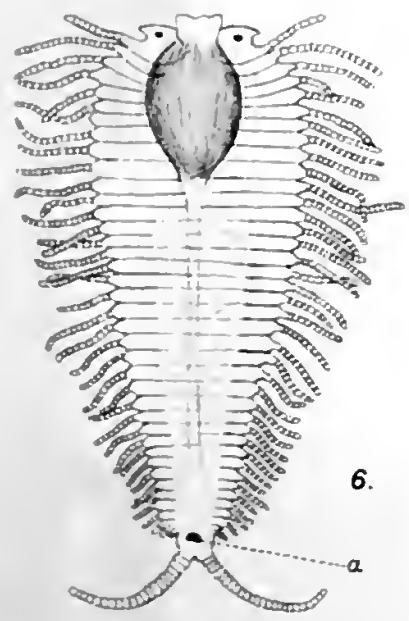
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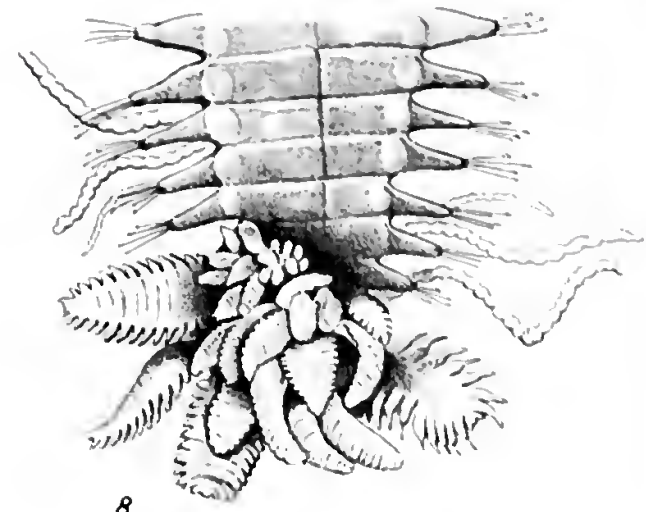
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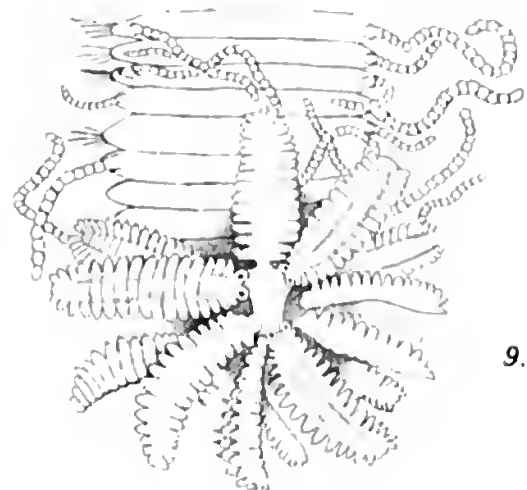
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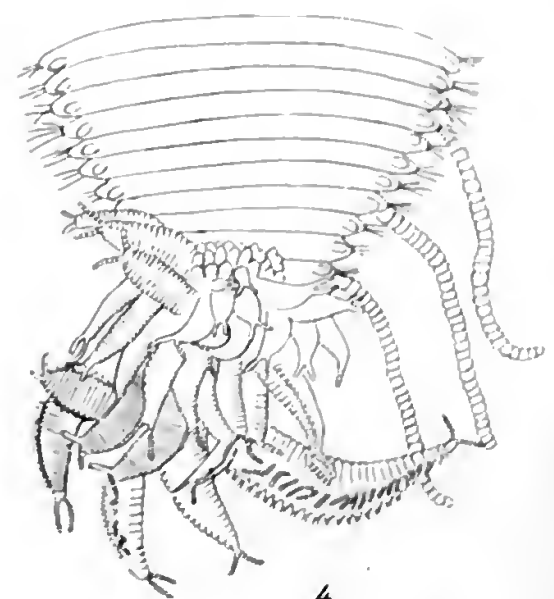
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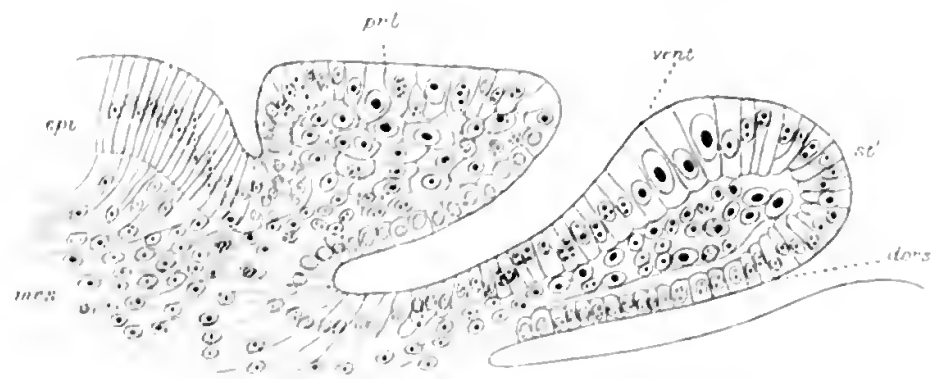
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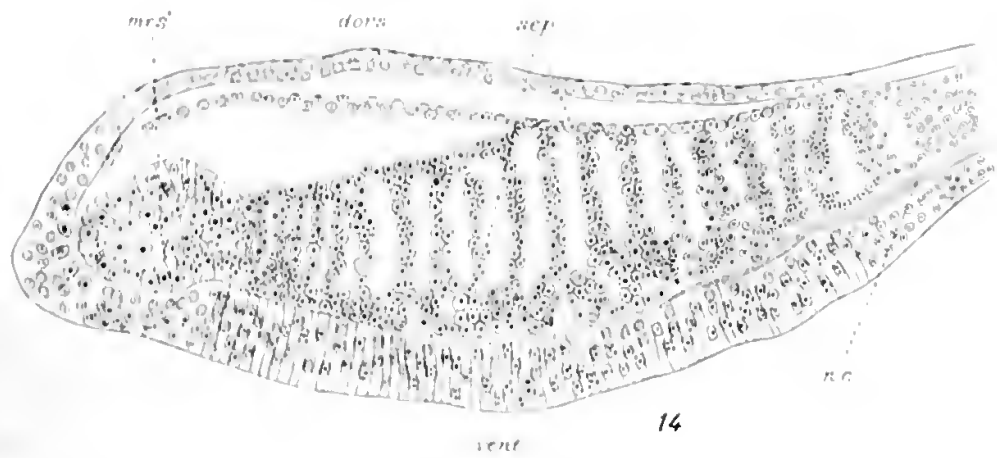




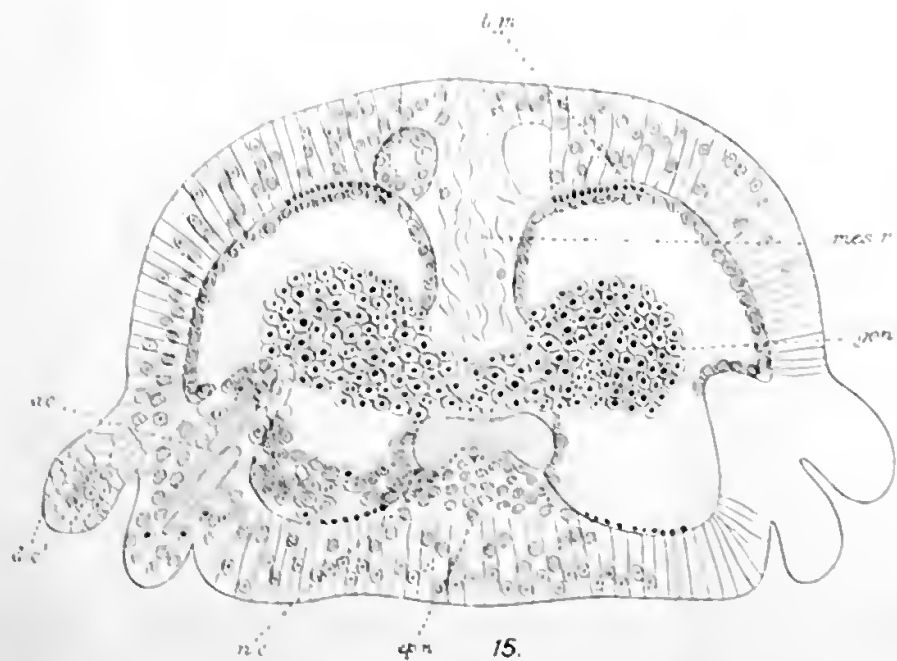




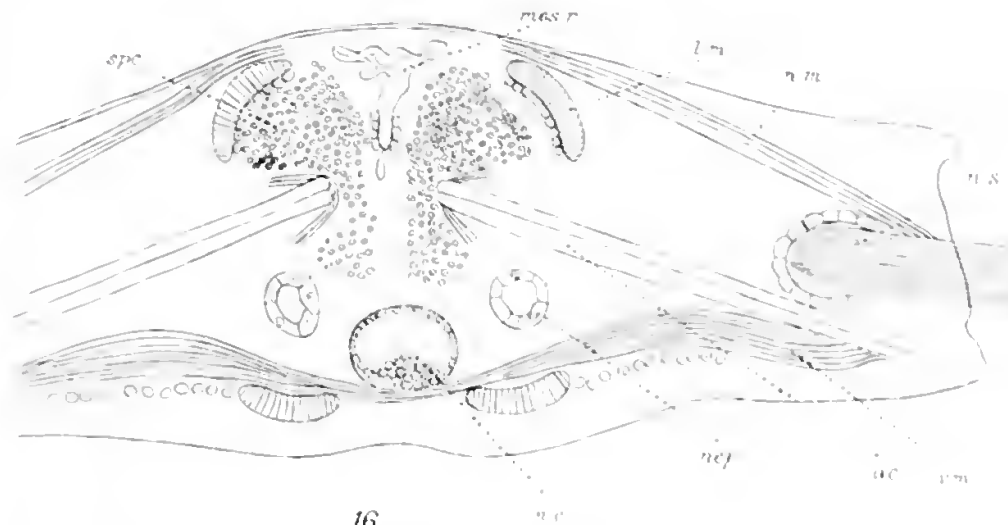
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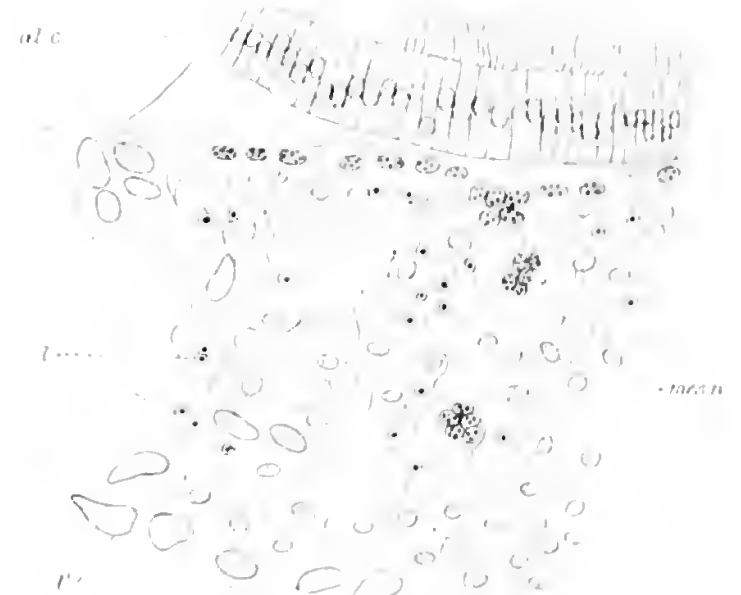
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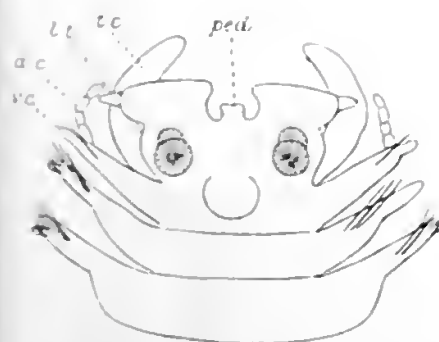
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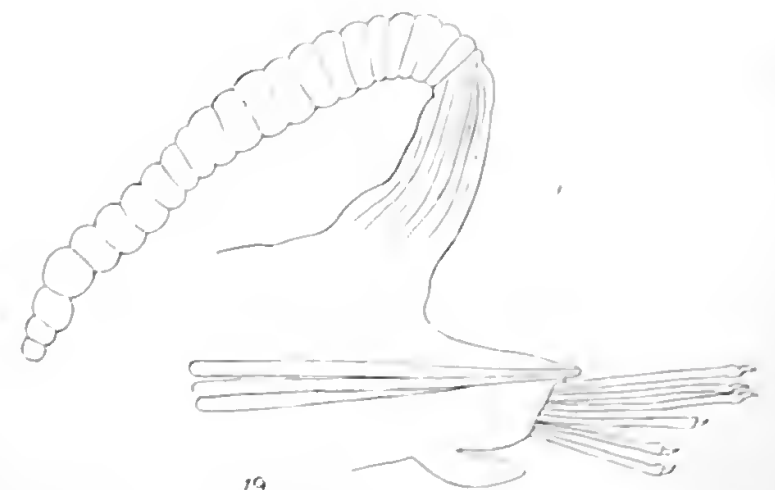
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# The Effects of Hypertonic Solutions upon the Fertilised Eggs of *Echinus* (*E. esculentus* and *E. acutus*).

By

**J. Gray, B.A.**

Scholar of King's College, Cambridge.

(From the Laboratory of the Marine Biological Association, Plymouth.)

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With Plates 24 to 27 and 4 Text-figs.

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IN the 'Proceedings of the Cambridge Philosophical Society,' vol. xvi, part v, 1911, a preliminary account was given of the cytology of the eggs from which the various hybrids obtained from the three species of *Echinus*—*E. esculentus*, *E. acutus*, and *E. miliaris*, have been reared by Shearer, De Morgan and Fuchs (15).

It was shown that whereas the first segmentation division of an egg of *E. esculentus* fertilised by the sperm of *E. acutus* was quite normal, that of the reverse cross showed certain abnormalities.

“Until immediately after the dissolution of the nuclear membrane in the first segmentation division the behaviour is normal, and thirty-eight normal chromosomes can be counted. As the spindle is formed, the chromosomes become scattered upon it irregularly, and gradually become collected in the equatorial plate. During this process it is seen that a considerable though variable number of them are either swollen up, or more commonly bear vesicles attached to their ends or sides. The staining of the vesicles is always less intense than that of the chromosomes, and is progressively fainter the more the vesicle is developed, so giving the impression that the chromosome has swollen at one point, and that the chromatin is thus more thinly diffused in the wall of the vesicle than in the normal part of the chromosome. In the equatorial plate stage the vesicles may either remain attached to the chromosomes which produced them, or become separated from them; those which become separated tend to take up positions round the edge of the equatorial plate, sometimes outside the spindle. The normal chromosomes and those of which the normal shape has not become much altered by vesicle production then split longitudinally in the ordinary way, and begin to travel to the poles. It may sometimes be seen that a chromosome with a vesicle attached has split, and the vesicle, remaining attached to one half, is being carried with it towards the pole. It is possible that a few chromosomes, the greater part of which has become swollen into a vesicle, do not divide, but are carried entire to one or other pole. The vesicles which have become separated from their parent chromosomes appear to differ in their fate according to their position. If they lie among the chromosomes inside the spindle, they are carried with them to one or other pole and become included in the daughter-nuclei. If, however, they are left on the edge of the spindle, as commonly happens with the larger vesicles, they remain outside the mitotic figure in the cytoplasm, and are not included in the nuclei of the daughter-cells. In this case they usually contract and become small evenly stained spheres, not easily distinguish-

able from the larger yolk-granules, but usually recognisable after the cell-division is completed, lying in the cytoplasm near the boundary between the two cells.

"In the second segmentation division a similar process takes place, but is usually rather less pronounced, the vesicles are on the whole smaller, and we doubt whether complete chromosomes ever become vesicular."<sup>1</sup>

A similar phenomenon within the eggs of Echinoderms has recently been described by Konopacki (7). He treated eggs of *Strongylocentrotus lividus* sixty minutes after fertilisation with hypertonic solutions (e. g. 50 c.c. sea-water + 9.5 c.c. 2½ M. NaCl solution) for half an hour, and subsequently transferred them to sea-water. His figures show structures somewhat similar to those found in the hybrid eggs of *Echinus esculentus* ♂ × *E. acutus* ♀. It was a study of these figures which led to the suggestion that the effect of hypertonic solutions upon the fertilised eggs of *Echinus esculentus* and *E. acutus* would throw some light on the formation of the vesicles found in the above-mentioned cross.

## II. METHODS.

Ripe eggs were fertilised in finger-bowls, and left for one hour. They were then transferred to hypertonic sea-water for half an hour, and subsequently put back into normal sea-water. The following strengths of hypertonic solutions were used.

### (1) For Eggs of *E. esculentus*.

Series A.	50 c.c. sea-water + 5	c.c. 2½ M. NaCl solution for ½ hour.
.. B.	50 c.c. .. + 6	c.c. .. .. .. ..
.. C.	50 c.c. .. + 7	c.c. .. .. .. ..
.. D.	50 c.c. .. + 8	c.c. .. .. .. ..
.. E.	50 c.c. .. + 9.5	c.c. .. .. .. ..
.. F.	50 c.c. .. + 20	c.c. .. .. .. 15 min.
.. G.	50 c.c. .. + 20	c.c. .. .. .. ½ hour.

<sup>1</sup> Quoted from 'Proc. Camb. Phil. Soc.,' vol. xvi, pt. V, 1911, p. 415.

(2) For Eggs of *E. acutus*.<sup>1</sup>

Series A.	50 c.c. sea-water	+ 5	c.c. 2½ M. NaCl solution	for ½ hour.
„ B.	50 c.c.	„	+ 6 c.c. „ „ „	„
„ C.	50 c.c.	„	+ 7 c.c. „ „ „	„
„ D.	50 c.c.	„	+ 8 c.c. „ „ „	„
„ E.	50 c.c.	„	+ 10 c.c. „ „ „	„
„ F.	50 c.c.	„	+ 15 c.c. „ „ „	„

The best fixative for these eggs has been found to be a mixture of corrosive sublimate and acetic acid (saturated solution of corrosive sublimate in sea-water 95 parts, glacial acetic 5 parts), and this has been used throughout. Sections of the eggs were stained with Heidenhain's iron-hæmatoxylin, other stains only being used for investigating particular structures.

## III. DESCRIPTIVE.

(1) The Effects of Hypertonic Solutions upon the Eggs of *E. esculentus*.

Series A.—Eggs were treated with 50 c.c. sea-water + 5 c.c. 2½ M. NaCl solution for half an hour.

The division figures for the large majority of these eggs are quite normal for the first segmentation. Only a few exceptions were found in which abnormalities could be detected. Fig. 1 shows a nucleus in which there is a distinct

<sup>1</sup> A comparison of the effects of various strengths of hypertonic solutions upon eggs derived from the same female shows an apparent inconsistency in the degree of abnormality caused by these solutions in respect to their strength: for example, series C of the eggs of *E. acutus* appears to be less affected in some way than the series B, although treated with a stronger solution. As, however, the exact way in which such treatment affects the chromatin is unknown, it is impossible to say whether this is due to the specific strength of the solutions used, or to more general causes. Again, in some cases the individual properties of an egg appear to determine to what extent it is affected by abnormal treatment; this is well seen in series C of the eggs of *E. esculentus*, in which different mitotic figures show a very wide range in their degree of abnormality.

vesicle similar to those to be described later for other series of eggs, while fig. 2 shows a somewhat later stage in which there is a small rounded mass of chromatin lying within the nucleolus.

Later stages of these eggs, however, show a certain amount of abnormality. Irregular cleavage is not uncommon, and in some mitotic figures the chromosomes become scattered irregularly on the spindle (figs. 3 and 4). Fig. 5 shows a polyspermic egg; this also is a fairly common occurrence in this batch of eggs.

Apparently the effect of the weak hypertonic solution is not sufficient to affect the nuclear elements until later stages than the first segmentation division.

Series B.—Eggs treated with 50 c.c. sea-water + 6 c.c.  $\frac{5}{2}$  M. NaCl for half an hour.

Preparations of these eggs show that the effect of the hypertonic solution is (1) to induce polyspermy, and the production of tripolar and tetrapolar spindles, and (2) to produce abnormal chromatic bodies within the nucleus. The extra sperms are apparently derived from those which become attached to the fertilisation membrane which is extruded after the entrance of one spermatozoon. Figs. 6A and 6B show the male and female pronuclei of one egg; within the female nucleus is seen two very conspicuous bodies which will be referred to in this paper as "vesicles." These bodies are usually round or oval in outline, and stain progressively lighter from the periphery to the centre. In the male nucleus are seen four similar though very much smaller bodies. Fig. 7 shows the fusion of an accessory sperm nucleus with a zygote nucleus; within the latter there can be counted over thirty definite chromosomes, while in the former there are about half this number.<sup>1</sup> Within the larger nucleus are seen one very large vesicle and four smaller ones, while within the accessory nucleus are also two small vesicles.

These large vesicles are characteristic of the female pronucleus, and must be derived from the female element,

<sup>1</sup> The somatic number of chromosomes for both *Echinus esculentus* and *E. acutus* is 38.



because (1) they can always be found in the female pronucleus, and never in the male; (2) in eggs in which more than one sperm has entered only one large vesicle is found. Fig. 8 shows an egg with a fusion nucleus showing a conspicuous and very darkly stained vesicle, while in the accessory sperm nuclei no such large bodies are found (only small dark dots among the chromosomes).

These vesicles are visible within the nuclear membrane before the chromosomes begin to be formed (fig. 6). They appear to arise from the network of the nucleus in the following manner: the outline of the vesicle is first distinguishable as a circle of dots enclosing minute granules of a darker staining substance. As the network of the nucleus becomes darker, preparatory to the formation of the chromosomes (which in these eggs arise as very fine crinkled threads), the interior of the vesicles gradually becomes more uniform, and it appears as though the granules gradually diffuse themselves until the whole vesicle is of a homogeneous nature. Fig. 9 shows a vesicle in the process of formation. In fig. 10 the vesicles are in such a condition as they are usually found in the later phases of division. Fig. 11 shows a nucleus as it is seen just before the nuclear membrane disappears: thirty-six or more chromosomes are present together with a large and a small vesicle. In mitotic phases of a later stage all the vesicles except the large one disappear, or can be only recognised among the chromosomes as small dark dots. The large vesicle, however, persists. Figs. 12-15 show the vesicle during the metaphase, anaphase, and telophase conditions of the spindle.

The ultimate fate of the large vesicle appears to vary with its position; if it lies on the centre of the spindle towards one pole, it passes up with the chromosomes and is included in the daughter-nucleus (fig. 14). More frequently, however, it lies towards the equator of the spindle; in this case it is omitted from both daughter-nuclei (fig. 15). Such omitted vesicles shrink to dark dots and can sometimes be detected near the cell-wall of 2-celled stages (fig. 16).

In the daughter-nuclei of the first division there are usually a few minute vesicles such as have been described for the male pronucleus—the fate and origin of these is unknown (fig. 16).

Fig. 17 shows that there is a tendency for some chromosomes to be omitted in the first division—the significance of this will be discussed later.

In tripolar and tetrapolar spindles, which are often derived from a female pronucleus which has fused with more than one male pronucleus, there is only one vesicle (figs. 17 and 17 A).

The interest of these vesicles lies in the fact that they are exactly similar both in appearance and behaviour to those which have been described (Doncaster and Gray [2]) for the hybrid eggs of the cross *E. esculentus* ♂ × *E. acutus* ♀. There is, however, one point of difference: in the hybrid eggs vesicles were never found earlier than the prophase of the first division, i. e. until after the nuclear membrane had disappeared; while in the eggs treated with hypertonic solution the vesicles are more numerous and distinct within the nuclear membrane than in any other phase of division.

The origin of the vesicles in the hybrid eggs is described in the introduction to this paper, and is obviously different to that of the vesicles in those eggs of *E. esculentus* which have been treated with hypertonic solutions.

Considerable time has been spent in an attempt to determine by actual chromosome counts whether the large vesicle in these hypertonic eggs is morphologically equivalent to one chromosome. This has not been altogether successful, as the crowding of the chromosomes makes it exceedingly difficult to determine whether their number is thirty-seven or thirty-eight. As, however, this vesicle is exactly similar in appearance and behaviour to those formed directly from the chromosomes, both in the hybrid eggs and in “hypertonic” eggs of *E. acutus*, I believe that this vesicle does represent a chromosome, and therefore regard it as being formed from that part of the nuclear network which normally goes to form

a particular chromosome ; the smaller vesicles would represent parts of such complexes—the remainder of which have gone to form chromosomes in the usual way.

The staining reactions of these vesicles are peculiar. They stain blue-black in Heidenhain's hæmatoxylin, dark blue in Ehrlich's hæmatoxylin, and maroon in Ehrlich-Biondi-Heidenhain. Whether they are to be regarded as chromatic or nuclear in nature is more fully discussed in another place.

Series C.—Eggs of *E. esculentus* treated with 50 c.c. sea-water + 7 c.c.  $2\frac{1}{2}$  M. NaCl solution.

The effect of a solution of this strength upon the chromatin varies a great deal for individual eggs: a more or less complete series can be found between eggs with normal mitotic figures, and eggs in which the chromatic complex is entirely disorganised.

Fig. 18 shows an advanced anaphase, in which the individual chromosomes are clearly visible, each possessing its characteristic shape ; the figure is, however, somewhat irregular, and the chromosomes, instead of being arranged in two definite plates are considerably scattered on the spindle towards the two poles. Two chromosomes are seen lying entirely off the mantle fibres.

Fig. 19 shows another anaphase which is slightly more irregular, but in which most of the chromosomes have retained their individuality. They have moved towards the poles in an irregular manner, the lower group especially being very irregular. In both groups it is seen that a few chromosomes have lost their normal shape, and have swollen up to form rounded or irregular masses of chromatin ; as in fig. 18, a few chromosomes have separated from the main spindle. Fig. 62 shows the two ends of an anaphase in face view ; nearly all the chromosomes are swollen, and in the right hand group is a large irregular mass of chromatin. Fig. 20 shows a prophase condition which apparently preceded such an anaphase as is shown in Fig. 19 ; the aggregation of some of the chromosomes into clumps is very characteristic.

Fig. 21 shows a still more irregular anaphase. In this case the whole mitotic figure has been affected. There is no trace of asters or spindle-fibres, all that can indicate their existence being a region of slightly denser and more darkly staining protoplasm round the chromatin groups.

The effect of the hypertonic solution upon the centrosomes is to cause them to become granular and to contract. In normal eggs the area of the centrosome is comparatively large and vesicular, as has been figured by Boveri and many other authors. They do not stain with iron-haematoxylin, and are quite free from granules. The centrosomes of fig. 18 are somewhat granular and more darkly stained than is normal. The extreme effect of the hypertonic solutions, however, is to cause the centrosomes to contract into compact but irregular masses, which stain as darkly as the chromatin in the nucleus (fig. 21, etc.) (Fig. 33 shows the contracted centrosomes still at the centre of normal asters for series G.) The chromosome groups of fig. 21 are shown enlarged in fig. 63. It will be seen that the centrosomes are entirely degenerated, although the chromosomes are only somewhat swollen.

Extreme cases of the degeneration of the nuclear structures is shown in figs. 22, 24. In fig. 23 a few chromosomes can still be detected as rod-like bodies, although this is somewhat doubtful; while in fig. 22 all that remains of the numerous rod-like and V-shaped chromosomes of the normal egg is represented by irregular masses of chromatin.

Fig. 25 shows a telophase in which all trace of the achromatic parts of the nucleus have disappeared. At each polar area is a group of normal chromosome vesicles arranged as in a normal egg. Within the lower group is seen a darkly stained vesicle which is exactly comparable to those found in series B, and to those found in the hybrid eggs of *Echinus esculentus* ♂ × *E. acutus* ♀. Outside each group of vesicles are irregular darkly stained masses, which apparently represent the degenerated centrosomes. In spite of the entire loss of the individual nature of the chromosomes

the chromatin is still able to form the normal chromatic vesicles in the telophase—in other words, such a stage as fig. 21 or fig. 23 is followed by that shown in fig. 25.

Text-fig. 1 shows two chromosome groups of the same spindle in which the chromosomes are apparently all normal with the exception of one which lies outside the main group and which is somewhat vesicular. It will be noticed that in one group there are 39 chromosomes and one vesicle (?) while in the other there are only 34 chromosomes. Now it has been shown (Doncaster and Gray) that the normal number of chromosomes is 38 for each group—a total of 76 for the whole:

TEXT-FIG. 1.



Two chromosome groups of the same spindle of an egg of *E. esculentus*, series C. In the left-hand group are thirty-four chromosomes, and in the right thirty-nine plus a dot.

this spindle only shows 73 to 75; the latter number if the chromatin body lying outside the two main groups represents a swollen chromosome which has failed to divide and has not been attracted to either pole. This shows that, owing to irregularity in the earlier stages of the division, both halves of a certain number of chromosomes must have passed to one and the same pole. This may be due to the fact that when division took place the mother chromosomes were lying abnormally nearer to one pole than to the other, so that both halves went to the same pole.

That irregular distribution of chromatin between the two poles does take place is seen in many of the more irregular

figures. These frequently show very much more chromatin aggregated at one pole than at the other.

Fig. 26 shows the nucleus of a 2-celled stage; it contains two minute vesicular bodies which are found in such nuclei in other batches of "hypertonic" eggs.

In spite of the profound disorganisation of the mitotic figures in this series of eggs, it is remarkable that in all the 2-celled stages examined, not a single case of irregular cleavage was observed. This is quite in accordance with the work of Paula Hertwig (6) on the effects of radium emanations on the eggs of *Ascaris megalocephala*. She has shown that eggs subjected to such treatment undergo chromatic degeneration in all respects similar to these eggs which were treated with hypertonic solutions; the mitotic figures disappear, the individuality of the chromosomes is lost, and the chromatin becomes aggregated towards each pole in irregular masses. She finds, however, that the first cleavage is perfectly regular (cf. her figs. 1-12).

The structures found in this series of eggs are interesting in two respects: (1) they partially explain why eggs of *Echinus acutus* when fertilised by *Echinus esculentus* sperm develop normally, although considerable irregularities occur within their chromosome complexes; (2) they appear to indicate that cleavage of the egg is not primarily dependent on the existence of the mitotic figure. The chromatin appears to segregate into two groups, and the egg divides normally without the existence of asters. As Paula Hertwig points out, cleavage appears to be a function of the cytoplasm, and is only indirectly controlled by the mitotic figure.

Series D.: *E. esculentus*.—Eggs treated with 50 c.c. seawater + 8 c.c.  $2\frac{1}{2}$  M. NaCl for 30 minutes.

Very few mitotic figures were found and no very definite statements can be made. Figs. 27-29 show nuclear figures of these eggs. It would appear as though the stage at which the chromosomes become visible as distinct units within the nuclear membrane is considerably delayed, and in many cases is never reached; owing, however, to the scarcity of good



preparations this cannot be insisted upon. Whether it is legitimate to compare these figures with figs. 23 and 25 of G. Hertwig (5) is doubtful. In no case were "vesicles" found within the nuclear membrane of these eggs (cf. fig. 30).

Series E.: *E. esculentus*.—Eggs treated with 50 c.c. sea-water +  $9\frac{1}{2}$  c.c. M. NaCl solution.

Fig. 31 shows an extreme effect of hypertonic solutions on an egg. Similar figures were obtained for first segmentation divisions. This is exactly comparable to Konopacki's (7) results on the eggs of *Echinus microtubercutus* treated with a slightly weaker solution (cf. his fig. 18).

Whether we are justified in assuming that the first mitotic figure of the particular egg which is figured was similar to those of the second division is doubtful, for it is possible that the pathological effect of the hypertonic solution did not affect the chromatin until the first division was over.

Konopacki assumes that the dark streaks are entirely chromatic in nature, but my preparations tend to show that part of the darkly stained material is derived from the spindle fibres.

Series F.: *E. esculentus*.—Eggs treated with 50 c.c. sea-water + 20 c.c.  $2\frac{1}{2}$  M. NaCl solution for fifteen minutes.

This batch showed very few mitotic figures, most of the eggs having evidently failed to develop. The figures available showed that, as a whole, division is normal although there is a tendency for the omission of one or more chromosomes (fig. 32).

Series G.: *E. esculentus*.—Eggs treated with 50 c.c. sea-water + 20 c.c. M. NaCl solution for 30 minutes.

In these eggs the hypertonic solution has visibly affected both the chromatin and the cytoplasm. Very few eggs develop, and those which do so show some sign of cytolytic action. Figs. 33 and 33 A show a 2-celled stage in which the first division of the egg was normal, but which was then preparing to divide very irregularly. There is a tendency for omission of chromosomes from the spindles in this egg. The centrosomes are contracted to small and compact bodies which stain darkly with Heidenhain's hæmatoxylin.



(2) Effects of Hypertonic Solutions upon the Eggs of *E. acutus*.

Series B.—Eggs treated one hour after fertilisation with 50 c.c. sea-water + 6 c.c.  $2\frac{1}{2}$  M. solution for 30 minutes.

This series of eggs shows a marked difference to the eggs of *E. esculentus* which were subjected to exactly similar treatment. Whereas in *E. esculentus* there was always one definite vesicle to be found in each nucleus, in *E. acutus* there are several. These are exactly similar in appearance and behaviour in the two species, being either omitted or included in the daughter-nuclei of the first segmentation division. As a rule the vesicles are either four or five in number for each mitotic figure, and one or two of them are conspicuously larger than the rest. This, however, is not an invariable rule, as figures have been found with four large vesicles all about the same size.

Like those found in the corresponding series of *E. esculentus* eggs, these vesicles stain pink in Erhlich-Biondi-Hedenhain's stain. Their origin in these eggs is, however, different. Numerous fused male and female pro-nuclei have been examined without detection of vesicles (fig. 34); on the other hand, in a few such nuclei well-formed vesicles are present (fig. 35). As, however, every astral figure seen (and these were very numerous) possessed well-defined vesicles, we cannot well attribute the origin of all these from the reticulum inside the nuclear membrane.

Fig. 47 shows individual vesicles considerably magnified, and their shape, together with the fact that many intermediate conditions between fully formed vesicles and normal chromosomes can be found, at once suggested that they have been formed in such a way as are the vesicles in the hybrid eggs *E. esculentus* ♂ × *E. acutus* ♀ (Doncaster and Gray [2]): in other words, it is absolutely certain that the majority of vesicles in these eggs are derived directly from the chromosomes. This being so, we should expect to find a reduction in the number of normal

chromosomes. There is no doubt that this is the case. Fig. 64 shows an anaphase in one group of which there are 34 chromosomes, and in the other 35—while between the two are two large vesicles, two chromosomes, and a small dot (the latter being larger than a normal chromosome and yet not definitely vesicular). Now if each of these large vesicles represents one of the 38 chromosomes of the original nucleus—then the total number of potential chromosomes would be  $34 + 35 + 7 = 76$ , which is the full number for an anaphase of a normal egg. Fig. 65 shows an aggregate of 76 in a similar way.

From figs. 36–45 it will be seen that the behaviour of these vesicles is exactly similar to those described for “hypertonic” *E. esculentus* eggs, and for the hybrid *E. esculentus* ♂ × *E. acutus* ♀. Fig. 40 shows the omission of two vesicles, and the inclusion of one vesicle among the chromosomes at each end of the spindle. A tripolar spindle with about seven vesicles is shown in fig. 48.

Figs. 49 and 50 show two aberrant nuclei comparable to G. Hertwig’s figs. 19–23 and 26, and Konopacki’s fig. 29.

If it could be shown that the vesicles in these eggs were always formed from the same individual chromosomes, there would be an interesting proof of the physiological individuality of these bodies. Unfortunately, however, treatment with a hypertonic solution of this strength often causes the normal shape of most of the chromosomes to be lost, so that the missing members of the complex cannot be identified by this means.<sup>1</sup> All that can be said, is that a certain number of chromosomes become converted into vesicles, from what elements in the male or female chromatin they are derived it is impossible to say.

It is just possible that those vesicles which are found within the nuclear membrane are really nucleoli, while those which are formed directly from the chromosomes must be regarded

<sup>1</sup> In the normal complex there can be identified two rod-like chromosomes which are distinctly longer than the rest; in these “hypertonic” eggs only one such chromosome can be recognised, but, as shown in the text, this evidence cannot be regarded as very satisfactory.

as essentially chromatic in nature. If this be so, however, it is remarkable that two nuclear elements of such widely different origin should appear so exactly similar. It seems more probable that those vesicles which are formed within the nuclear membrane are formed from those parts of the network which normally enter into the composition of one or more chromosomes, as is suggested elsewhere in this paper.

Series C.—*E. acutus* eggs treated with 50 c.c. sea-water + 7 c.c.  $2\frac{1}{2}$  M. NaCl solution.

In preparations of these eggs no vesicles have been found. The chromosomes are, however, somewhat irregular, as they show a marked tendency to group themselves together. On account of this, accurate determinations of their number is usually impossible, but in some case the full somatic number can be counted. In anaphase plates it is remarkable that many of the chromosomes appear double (cf. fig. 67). Usually the mitoses are somewhat irregular, and in some cases there is a marked omission of chromosomes from the main groups, as shown in figs. 67 and 68. Fig. 69 shows a condition very similar to Baltzer's (1) figs. 25 B and 33 A of the first segmentation division of *Sphærechinus* ♂ × *Strongylocentrotus* ♀ and the second division spindle of *Echinus* ♀ × *Sphærechinus* ♂ respectively.

Another peculiarity in these eggs is that the spindle is very much narrower than is normal for this species of *Echinus* (fig. 51), in fact the spindle in this series of eggs recalls the narrow spindle of the hybrid *E. miliaris* ♂ × *E. acutus* ♀. Is it possible that the narrow spindles of this hybrid are a result of abnormal conditions due to hybridisation, and are not a characteristic feature of the male parent? (The spindles of *E. miliaris* ♀ × *E. miliaris* ♂ are broader than those of the hybrid eggs.)

Series F.—Eggs treated with 50 c.c. sea-water + 15 c.c.  $2\frac{1}{2}$  M. NaCl solution.

In this series of eggs the process of vesicle formation is still more pronounced than in series B. There are, however, one or two points of distinction between these series. Practi-

cally all the chromosomes in some eggs of series F show a distinct tendency to form vesicles (figs. 56-60). Every gradation is found between typical rod-like chromosomes and large well-formed vesicles, and there can be, therefore, no doubt whatever that these bodies are formed from the chromosomes after the nuclear membrane breaks up. This is substantiated by the fact that it is only the exception to find vesicles within the membrane of the fused male and female pronuclei. Fig. 53 shows such an exception.

Besides the formation of numerous vesicles many mitoses show that there is a tendency for the chromosomes to be aggregated together in clumps, in some cases giving rise to irregular masses of chromatin (fig. 58).

Chromosome counts in the anaphases of these eggs, shows that the formation of the vesicles has reduced the number of the chromosomes, as would be expected from their mode of formation. In all cases counted it was found that 32 to 34 was the average number for each group, together with a varying number of vesicles. If we regard, as before, each vesicle as equivalent to a chromosome removed from each group of the anaphase in which it is found, then the number of chromosomes present, plus twice the number of vesicles, should give 76 units (cf. figs. 70, 71). This is sometimes the case, but numerous exceptions have been found, in which the aggregate is only 74 or less. But as in many cases the large vesicles in anaphases are exceedingly lightly stained (cf. fig. 61), it seems reasonable to suppose that in some cases they disappear altogether, the only evidence of their previous existence being the reduced number of the chromosomes. It is also possible that one vesicle may be formed from more than one chromosome, as is suggested by the shape of the vesicle in fig. 53 (cf. also the last vesicle fig. 47).<sup>1</sup>

<sup>1</sup> The low value of some of these chromosome counts is doubtless to be explained by wrong interpretation as a single unit, of what is really two chromosomes closely approximated together. This approximation of chromosomes is characteristic of this series of eggs.

Fig. 52 shows the normal condition of a nucleus before the break-up of the nuclear membrane. Thirty-eight chromosomes can be identified, although the exact number is often difficult to determine, owing to portions of individual chromosomes occurring in two sections of the same nucleus. There are typically no vesicles. Just as in the other series of eggs, however, exceptions are found (figs. 53-55). The large vesicle in fig. 53 has a peculiar structure, and which, as is suggested above, may have arisen from more than one centre, therefore, being equivalent to more than one chromosome.

Fig. 61 shows an anaphase, with the chromosomes sometimes very irregular in outline. In the centre of the spindle is a large vesicle, the interior of which is practically unstained, while round its periphery are a number of minute dark dots.

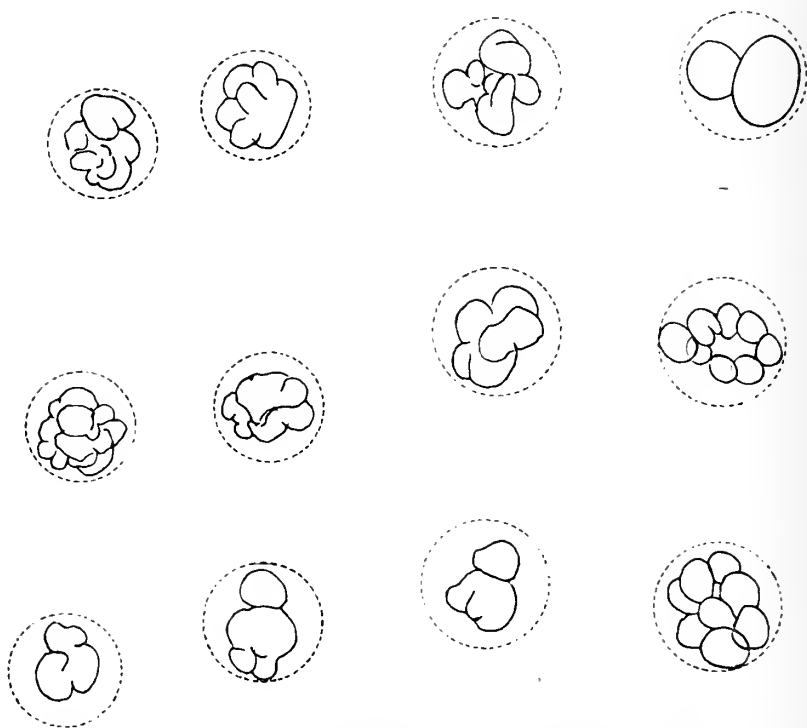
#### Later Stages in the Development of "Hypertonic" Eggs.

The eggs of *E. esculentus* which have been treated with hypertonic solutions of moderate strength usually behave quite normally until after the first cleavage has been completed; only a few exceptions were found in which the first two blastomeres were unequal. After this, segmentation usually becomes irregular, and gives rise to blastulæ such as are figured (Text-fig. 3). Great mortality occurs at this stage; only a few gastrulæ were obtained, and these were more or less abnormal and never gave rise to plutei.

"Hypertonic" eggs of *E. acutus* show a somewhat similar development. Text-fig. 4 represents irregular blastulæ from these cultures. The development is retarded considerably by treatment with the solutions. The first cleavage is normal in all the eggs which develop, even in the case of eggs treated with 50 c.c. sea-water + 15 c.c.  $2\frac{1}{2}$  M. NaCl solution. The later segmentation of such eggs is irregular in all the cultures; and the number of these abnormalities varies roughly from 1 to 20 per cent. of the total number of

eggs according to the strength of hypertonic solution with which they have been treated. The blastulæ derived from these irregular eggs are all abnormal (Text-fig. 4). Probably none of them develop further. Some, however, appear to be

TEXT-FIG. 2.

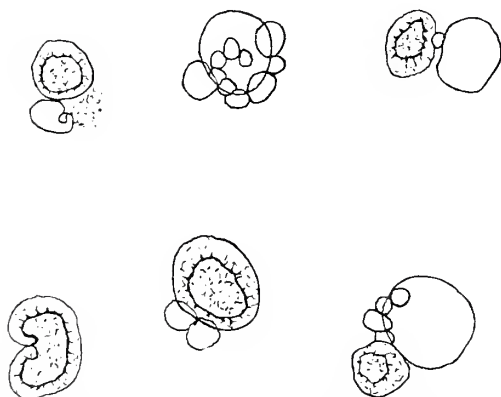


Eggs of *E. esculentus* which had been treated with hypertonic seawater for half-an-hour. Four hours after fertilisation.

“half blastulæ,” i. e. only one side of them is irregular. At a later stage only healthy plutei are found, and these have developed from eggs whose segmentation was normal.

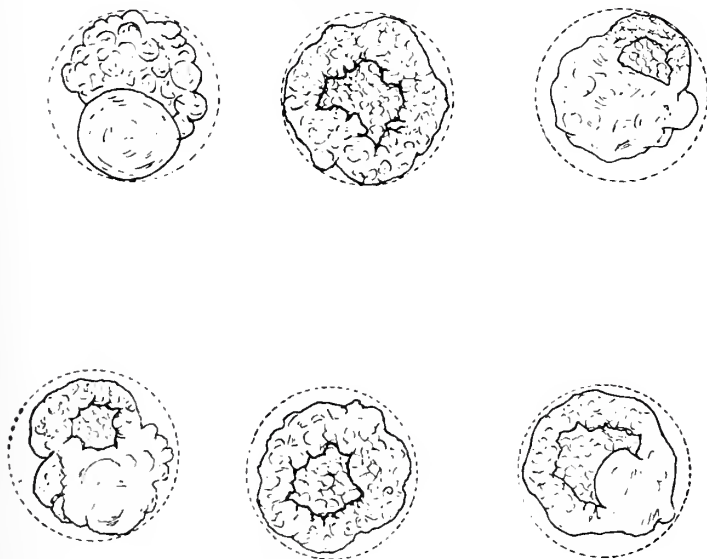
There are also, however, a few plutei half the normal size—apparently these have developed from the stereo-blastulæ.

TEXT-FIG. 3.



Eggs of *E. esculentus* which had been treated with hypertonic sea-water. Seventeen hours after fertilisation.

TEXT-FIG. 4.



Blastulae reared from eggs of *E. acutus* which had been treated with hypertonic sea-water for half-an-hour. Seventeen hours after fertilisation.



## IV. SUMMARY OF EXPERIMENTAL WORK.

(1) The chromatin of *Echinus acutus* under certain abnormal conditions behave differently to that of *E. esculentus* which is subjected to similar conditions.

(2) The effect of hypertonic solutions of a certain strength on the fertilised eggs of *E. acutus* is to cause the elimination of chromosomes from the nuclei by a process exactly similar to that which is normally found in the hybrid eggs of the cross *E. esculentus* ♂ × *E. acutus* ♀. This phenomenon cannot be induced in the fertilised eggs of *E. esculentus* by similar treatment.

(3) In eggs of which the whole nuclear structure has been disorganised, the first segmentation division is normal.

(4) The later segmentation of all eggs which have been treated with hypertonic solutions is abnormal.

## V. COMPARISON OF RESULTS WITH THOSE OF OTHER INVESTIGATORS.

In 1903 Teichmann (17) published an account of the cytology of the eggs of *Echinus microtuberculatus* fertilised by the sperm of the same species, which had been treated with .05 per cent. solution of strychnine previous to use. He showed that the male chromatin failed to become active until after the first segmentation division, during which period it can be seen lying near to the spindle as a compact chromatic body. Later on it becomes active and amalgamates itself with the female chromatin of one of the blastomeres.

In 1911 Konopacki (7) described the effect of hypertonic solutions upon the fertilised eggs of *Strongylocentrotus lividus* and *Echinus microtuberculatus*. Although a detailed description of the chromatin is not given, it may be concluded from his figures that his results were similar to those described in this paper for *Echinus esculentus* and *E. acutus*. Figs. 1, 2 of Konopacki's work show irregular anaphases in *Echinus* eggs which have been treated for half an

hour with 50 c.c. sea-water + 5 c.c.  $2\frac{1}{2}$  M. NaCl solution, similar to figs. 3 and 4 of this work. Figs. 46 and 38 of his paper show the swelling of the chromosomes, while figs. 37 and 39-50 make it appear as though a process of vesicle-formation had taken place, comparable to what I have described. Fig. 31 of this paper is quite comparable to figs. 14 and 18 of Konopacki. I have failed, however, to find structures similar to his figs. 17, 20, 24, 31-36. A comparison of the figures of the two papers will make these points apparent.

In a recent paper Günther Hertwig (5) has described the cytology of the eggs of *Parechinus miliaris* (*Echinus miliaris*) fertilised by sperm of the same species which had been previously subjected to radium emanations. As his results have some bearing on the general questions at issue, it may be convenient to give a summary of his work. In the case of eggs which were fertilised by sperm which had been treated with rays from a "Mesothoriumpräparat" (55 mg. pure radium bromide) for twelve to fifteen hours, the male chromatin was found to have lost its power of development and remained quiescent just as in Teichmann's preparations; it never regained its activity, however, and eventually degenerated in the cytoplasm of the egg.

In some of the figures which illustrate the fate of sperm chromatin which has been treated with "Radiumpräparat I" (7.4 mg. pure radium bromide) for sixteen hours, the male pronucleus is seen to have fused with that of the female, immediately giving rise to abnormalities. Hertwig describes his results as follows: ". . . zu einer Zeit, wo die Kontrolleier schon zweigeteilt waren, der Furchungskern sich etwas in die Länge gestreckt hat; an seinen beiden Enden sind schwache Strahlungen entwickelt, die Stelle, wo der Spermakern mit dem Eikern verschmolzen war, ist noch daran kenntlich, das hier eine dichtere Anhäufung von Chromatinfaden sich findet. Ferner sind, unabhängig von dem Spermachromatin, in der Masse des ursprünglichen Eikerns, (drei) mit Heidenhainschen Hämatoxylin sich tiefschwarz tingierende, mit Biondi-lösung sich ebenso wie

der gesammte Kern rot färbende Körner aufgetreten, die in ihrem Innern eine Art Vacuole aufweisen. . . . Ich glaube diese Körnchen als Nucleolen deuten zu dürfen."

There can be little doubt that these "Körnchen" are to be identified with the "vesicles" found within the nuclear membrane of the eggs of *E. esculentus* and *E. acutus*, which have been treated with hypertonic sea-water.

Now, in series B of the eggs of *E. acutus*, it has been shown that the formation of the vesicles is attended by a reduction in the number of the chromosomes, and yet these vesicles do not show a chromatic reaction with Ehrlich-Biondi-Heidenhain's stain. This is, however, not a conclusive proof that the vesicles are not derived from the chromosomes, because the normal chromosome vesicles, which are formed directly from the chromosomes in the telophase of Echinoid eggs, are also stained pink by this reagent. Hence it seems justifiable to assume that the formation of the vesicles and the elimination of them from the rest of the chromatin of the nucleus is equivalent to the elimination of the chromosomes themselves, and that these vesicles are not necessarily of nucleolar origin—a conclusion which receives very strong support from the mode of formation of these vesicles themselves. Hertwig's fig. 24 makes it appear as though in some cases the nucleus divides into two without the disappearance of the nuclear membrane, by a process of "amitosis," such as Konopacki described (see Konopacki's figs. 31 and 33). In other cases (as when the sperm received lower intensities of radium treatment) the mitotic figures are more normal, a typical spindle and asters being formed. The chromatin in these cases, however, is seen to be quite abnormal, for, while some of the chromosomes are recognisable as rod-like bodies, a considerable number are represented by irregular masses of chromatin scattered on the spindles (which are often multipolar, as in the eggs of *E. esculentus* here described) (cf. his figs. 28-32). Most of this chromatin "scheint teils in Form der kleinen Körnchen vorhanden zu sein, teils hat es sich auch

im Plasma, das dadurch seine Färbbarkeit erhalten hat, wieder gelöst. Über die Herkunft der Intensiv gefärbten, grossen Klumpen können wir nur Vermutungen äussern; einmal werden sie wohl Reste von Nukleolarsubstanz enthalten, andererseits wäre auch daran zu denken, das zu ihrer Bildung das väterliche Radiumchromatin beigetragen hat."

Later stages of such eggs also resemble those of *E. acutus* which have been treated with hypertonic solutions, being characterised by very irregular segmentation and the production at times of stereoblastulae (cf. Hertwig's text-figs. 6, 7, 8).

Hertwig finds that the introduction of an abnormal sperm nucleus into the female pronucleus leads to abnormalities in the female chromatin. He concludes, however, that "diese Schädigung des mütterlichen Chromatins allein durch den radiumkranken Spermakern hervorgerufen sind," because the control eggs behaved normally. He does not entertain the idea that it is possible for the female cytoplasm to affect the female chromatin either after the break-up of the nuclear membrane or before. As a result of the work described in the present paper, however, this is a consideration which must be entertained, for, as the eggs in this case were not subjected to abnormal treatment until an hour after fertilisation, any irregularities in the nuclei must be attributed to the effects of the deranged cytoplasm of the egg.

## VI. HYBRIDISATION AND PATHOLOGY.

The primary effect of the treatment of the fertilised eggs of *E. acutus* with hypertonic solution of medium strength is to cause the elimination of a certain number of chromosomes from the nucleus. G. Hertwig's results show that this can be likewise effected by fertilising normal eggs with sperm which has been rendered abnormal. It may be concluded, therefore, that elimination of chromatin from the nucleus can be referred to pathological conditions of the nucleus itself, and is not exclusively a result of hybridisation.

In 1908 Baltzer showed that in hybrid eggs of the cross *Sphaerechinus granularis* ♂ × *Strongylocentrotus lividus* ♀ there is an elimination of fifteen chromosomes in the first two segmentation divisions. The eliminated chromosomes are found as double units lying at the equator of the spindle (c f. his text-figs. ii A and vii). This is a condition similar to that shown in fig. 69 of this paper. In the reverse cross Baltzer found no elimination; the cross was difficult to obtain, and, although most of the mitoses were normal, a few showed marked abnormalities. Text-fig. iii of his paper shows an irregularity in the nucleus which is strikingly similar to figs. 21-25 of G. Hertwig, except for external form. (See also fig. 50 of this paper.)

The elimination of chromosomes from hybrid eggs has also been described by Tennent (18 and 19). He has shown that in the cross *Toxopneustes* ♂ × *Hipponoë* ♀ there is a continuous elimination of chromosomes up to the 16-cell stage; while in the reciprocal cross no elimination occurs.

A comparison of the cytology of hybrid eggs with that of eggs rendered pathological before or after fertilisation shows a remarkable similarity between the two.

Kupelwieser (8) showed that when eggs of *Echinus microtuberculatus* are fertilised by the sperm of *Mytilus galloprovincialis* the male chromatin remains inactive, and does not fuse with the female pronucleus. This is exactly similar to what happens if such eggs are treated with pathological sperm of the same species (Teichmann), or when eggs of *Parechinus miliaris* are fertilised by sperm rendered abnormal by strong radio-active treatment (G. Hertwig); compare Kupelwieser fig. 19, and G. Hertwig fig. 6.

The characteristics of Baltzer's hybrid eggs are likewise an elimination of chromosomes from the nucleus, and in exceptional cases abnormalities within the nuclear membranes (text-fig. x, Baltzer).

As an appendix to his paper, Konopacki compares his results with those of Baltzer; he is of the opinion, however, that although the structures observed in the two cases are

remarkably similar, they are fundamentally different, as he never observed any elimination of chromatin from the nucleus.

In the introduction to this paper, a short description was given of the cytology of the hybrid eggs of *Echinus acutus* ♀ × *E. esculentus* ♂. It has now been shown that the phenomena observed in this cross can be exactly paralleled by the effects of hypertonic solutions upon the eggs of *E. acutus*, and that similar treatment affects *E. esculentus* in a different way.

A conclusion which may, therefore, be made is that the cytological phenomena of hybridisation are, in fact, the phenomena of pathology, and that, when an egg is fertilised by the sperm of a foreign species, the behaviour of the male and female chromatin tends to become irregular, owing to a general derangement of the whole nucleus, elimination, therefore, cannot be regarded as a simple rejection of that part of the nucleus which is out of tune with the rest, or with the cytoplasm of the egg.

In other words, we must assume that, whenever the condition of the nucleus becomes peculiarly abnormal, certain of its constituent elements are affected to such an extent that they are no longer able to pass through their normal phases, and, therefore, fail to take part in the further development of the cell; thus, in the case of the chromosomes, some are, apparently, more affected than others, and are eliminated from the nucleus.

The bearing of these results upon the cytology of the reciprocal hybrids of the species of *Echinus* is more fully described in another paper (Doncaster and Gray [2a]).

## VII. THEORETICAL.

Until recently, it was supposed that the cell-membranes in animal tissues were practically impermeable to electrolytes. It has now been shown that this is not the case, and considerable evidence is available to support the opinion that the



relation of the cell to electrolytes is of prime importance to its existence.

The work of McClendon (13) and R. S. Lillie (9-11) has shown that, after fertilisation, the egg surface is more permeable to ions than before. It has also been demonstrated that the  $\text{CO}_2$  production of a dividing cell, changes just before each division, i. e. there is a slight increase in permeability.<sup>1</sup> It is also highly probable that the effect of hyper-tonic solution upon a cell is to decrease its permeability to ions (Sutherland [16], Lillie).

The whole of the evidence in favour of the view that the permeability changes of the egg membranes (plasmic and nuclear) are of profound importance to the activities of the cell cannot be discussed in full, but the following extract from a recent paper by R. S. Lillie (11) has a peculiar interest in connection with the phenomena discussed in this paper:

"The conclusion that many pathological conditions have their primary origin in abnormalities of the limiting membranes of cells is an obvious corollary of any view that regards such membranes—which are essentially insulating surface films of varying ionic permeability and electrical polarisation—as largely controlling the rate and character of the cell processes. If stimulation depends primarily upon altered polarisation of the plasma-membrane due to increased ionic permeability, it is clear that a normal response in the case of any cell, implies a definite condition of the membrane. If this condition is permanently altered, the cell processes inevitably undergo derangement, and pathological changes follow. Such a deranged condition, if not too far advanced, may be rectified by restoring the membrane to its normal condition. . . . The alteration caused by a toxic agent may consist primarily either in increasing or in decreasing the permeability normal to the membrane, or in altering in either direction the readiness with which the latter undergoes

<sup>1</sup> The susceptibility of a dividing cell to poisons has also been shown to be rhythmical in the same way.



change. . . . The plasma membrane cannot undergo marked and prolonged increase of permeability without alteration in the nature and proportion of the cell-constituents; this involves altered chemical organisation and eventual derangement of the cell-processes" (pp. 344 and 345).

It is, therefore, a possibility that the hypertonic solutions of a fertilised egg exert a toxic action upon the nucleus by upsetting the normal relationship of the cytoplasm to electrolytic ions.

Now, the permeability of the egg is changed when spermatozoon enters, and presumably the change is constant in degree for each species. When, however, the sperm of a foreign species is made to enter an egg, is it not possible that the change in permeability is not that which would have been caused by a sperm of the species to which the egg belongs? If this be so, and the degree of change in permeability of an egg when fertilised is, therefore, a function of the sperm, then the cytological behaviour of reciprocal crosses is explicable.

Let the change in } permeability for }	E. acutus eggs { fertilised by }	E. acutus sperm be P
"	E. esculentus ..	E. esculentus .. $P_1$
Then "	E. esculentus ..	E. acutus is P
and "	E. acutus ..	E. esculentus is $P_1$

Let the difference between P and  $P_1$  be about equal to the change of permeability in normally fertilised eggs of E. acutus which is brought about by the action of hypertonic solutions of appropriate strength.

Now the chromatin of E. esculentus can withstand a change of permeability in the surrounding protoplasm equal to  $P - P_1$  without becoming abnormal,<sup>1</sup> as is shown by its reaction to a hypertonic solution capable of producing such a change. Hence when the egg of E. esculentus is

<sup>1</sup> Presumably the change  $P - P_1$  is a little less than that caused in eggs of E. esculentus by 50 c.c. sea-water + 6 c.c.  $2\frac{1}{2}$  M. NaCl, as in the latter case one vesicle is produced, while in the hybrid E. acutus ♂ × E. esculentus ♀ no such abnormality occurs.

fertilised by the sperm of *E. acutus*, and so attains the permeability peculiar to fertilised eggs of *E. acutus*, no abnormality occurs.

On the other hand, when the egg of *E. acutus* is fertilised by sperm of *E. esculentus*, the *E. acutus* element becomes pathological, as it cannot endure a permeability in its surrounding protoplasm equal to that characteristic of an egg of *E. esculentus*.

Such a change in permeability might well give rise to changed osmotic relations between the nucleus or chromosomes and cytoplasm, such as has been suggested (Doncaster and Gray [2a]), as the direct cause of vesicle formation in hybrid eggs.

This hypothesis implies that in the hybrids it is essentially the female chromatin which gives rise to the vesicles. It is impossible to determine this from a study of the hybrid eggs, but the effects of hypertonic solutions upon the eggs of the two species give strong support for such an assumption.

This suggestion is, however, in entire opposition to most of the conclusions of other workers on hybridisation. The work of Kupelwieser, Baltzer and Tennent all tends to show that it is the male chromatin which becomes abnormal in such cases. Tennent, however, has shown that in the crosses *Arbacia* × *Toxopneustes* not only are all the chromosomes which are derived from the male eliminated, but also some of those from the female parent. Again, G. Hertwig has shown that fusion with an abnormal sperm can cause the female chromatin to be affected.

Again, abnormalities are to be entirely confined to the male element in the egg, there is apparently no explanation for the cytology of such reciprocal crosses as *Sphærechinus* × *Strongylocentrotus*, or *Echinus acutus* × *E. esculentus*; for if, as Baltzer suggests, the male chromatin goes wrong because it is "out of tune" with the female cytoplasm, why are not both reciprocal crosses abnormal? And in the case of *E. acutus* ♂ × *E. esculentus* ♀ we should expect

the more sensitive *E. acutus* chromatin to go wrong when it enters the cytoplasm of the more resistant *E. esculentus*, and yet this is not the case.

The proof of the hypothesis, here put forward tentatively, would lie in the demonstration of the fact that the permeability change of an egg when fertilised differs according to the species of sperm used—in other words, the degree of permeability change should be a function of the sperm.<sup>1</sup>

In conclusion, I wish to express my sincere thanks to Mr. Doncaster for much valuable advice during the progress of this work, and to Dr. Shearer for his valuable help in the preparation of this paper for publication. I also wish to thank the officials of the Plymouth laboratory for their un-failing assistance in obtaining material.

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<sup>1</sup> As in some cases of hybridisation there is no doubt whatever that it is the male chromatin that becomes abnormal (e.g. Kupelwieser's experiments), it is obvious that changes of permeability of the cytoplasm, as can be induced by the sperm, cannot be a sufficient explanation of all the abnormalities observed in cross-fertilised eggs.

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## EXPLANATION OF PLATES 24-27.

Illustrating Mr. J. Gray's paper on "The Effects of Hypertonic Solutions upon the Fertilised Eggs of *Echinus*. (*Echinus esculentus* and *E. acutus*)."

[Most of the outlines of the figures were drawn at table level with a camera, under Zeiss ocular 2 and 2 mm. oil-immersion objective.]

## PLATE 24.

## Figs. 1-20.

Figs. 1-5.—Nuclear figures of eggs of *Echinus esculentus* which had been treated with 50 c.c. sea-water + 5 c.c.  $2\frac{1}{2}$  M. NaCl for half an hour.

Fig. 1.—Fused male and female pronuclei showing vesicle.

Fig. 2.—Fused male and female pronuclei showing no vesicle, but a small dark mass of chromatin.

Figs. 3 and 4.—Later stages of eggs in this series showing regular and irregular mitotic figures. [Magnification oc. 2, objective  $\frac{1}{6}$ .]

Fig. 5.—Egg showing numerous sperm nuclei in process of division. [Magnification oc. 2, objective  $\frac{1}{6}$ .]

Figs. 6-16.—Nuclear figures in eggs of *E. esculentus* which had been treated with 50 c.c. sea-water + 6 c.c.,  $2\frac{1}{2}$  M. NaCl for half an hour.

Figs. 6a and 6b.—The female and male pronuclei from a single egg. Within the former are two vesicles, while in the latter are four minute bodies with a faint vesicular nature.

Fig. 7.—Fusion of a large and small nucleus; the former contains thirty-six or more chromosomes, with one large and four small vesicles. The accessory male nucleus shows two small vesicles.

Fig. 8.—This egg shows two accessory male nuclei which have not fused with the female pronucleus. Each show the typical haploid number of chromosomes and no vesicles. Within the larger nuclei are the full somatic number of chromosomes, together with a large, very darkly stained vesicle.

Fig. 9.—Shows two large and two small vesicles within a nucleus whose chromosomes are well defined.

Fig. 10.—Shows the formation of a vesicle from the reticulum of the nucleus.

Fig. 11.—Prophase showing one large and one small vesicle.

Fig. 12.—Metaphase showing large, unpaired vesicle.

Fig. 13.—Anaphase showing omission of the vesicle.

Fig. 14.—Telophase with the vesicle appressed to one of the daughter-nuclei.

Fig. 15.—Similar stage with omission of the vesicle, which has now contracted to a dark dot.

Fig. 16.—Two-celled stage showing omitted chromatin between the nuclei. [Magnification oc. 2, objective  $\frac{1}{6}$ .]

Fig. 17.—Tripolar anaphase showing omission of one vesicle and several chromosomes.

Fig. 17 a.—Quadripolar figure showing a dispermic nucleus with one vesicle.

Figs. 18-26.—Nuclear figures in eggs of *E. esculentus* which had been treated with 50 c.c. sea-water + 7 c.c.  $2\frac{1}{2}$  M. NaCl for half an hour.

Fig. 18.—Anaphase with chromosomes arranged irregularly on the spindle.

Fig. 19.—Anaphase somewhat more irregular than fig. 18.

Fig. 20.—Irregular prophase without trace of asters or spindle.

## PLATE 25.

### Figs. 21-33 a.

Fig. 21.—Still more irregular anaphase: the centrosomes degenerated into irregular, darkly stained bodies, and the shapes of the chromosomes mostly lost.

Fig. 22.—The chromatic complex is represented by irregular masses of chromatin.

Figs. 23 and 24.—Very irregular anaphases.

Fig. 25.—Telophase with degenerated asters and centrosomes. Within one group of chromosome vesicles is a small irregular vesicle.

Fig. 26.—Nucleus of a 2-cell stage with two minute vesicles.

Figs. 27-30.—Nuclear figures in eggs of *E. esculentus* which had been treated with 50 c.c. sea-water + 8 c.c.  $2\frac{1}{2}$  M. NaCl solution for half an hour.

Fig. 31.—Egg of *E. esculentus* which was treated with 50 c.c.

sea-water + 9.5 c.c.  $2\frac{1}{2}$  M. NaCl for half an hour. The chromatin is entirely degenerated, but the asters are normal. [Magnification oc. 2, objective  $\frac{1}{6}$ .]

Fig. 32.—Late anaphase of an egg of *E. esculentus* treated with 50 c.c. sea-water + 20 c.c.  $2\frac{1}{2}$  M. NaCl for fifteen minutes. One chromosome has been eliminated.

Figs. 33 and 37 A.—Two sections of a segmenting egg treated with 50 c.c. sea-water + 20 c.c.  $2\frac{1}{2}$  m. NaCl for half an hour. The cytoplasm shows signs of cytolysis and the centrosomes are contracted. [Magnification oc. 2, objective  $\frac{1}{6}$ .]

#### PLATE 26.

##### Figs. 34-61.

Figs. 34-50.—Nuclear figures from eggs of *E. acutus* which had been treated with 50 c.c. sea-water + 6 c.c. of  $2\frac{1}{2}$  M. NaCl for half an hour.

Fig. 34.—Fused male and female pronucleus showing no vesicles.

Fig. 35.—Nucleus in same phase as fig. 34, but with vesicles.

Fig. 36.—Prophase showing four vesicles.

Fig. 37.—Metaphase showing seven vesicular elements.

Fig. 38.—Metaphase showing six vesicles, the shape of some indicate their origin from the centrosomes.

Fig. 39.—Metaphase showing five vesicles.

Fig. 40.—Anaphase showing two vesicles omitted, and one included in each chromosome group.

Fig. 41.—Anaphase showing three omitted vesicles.

Fig. 42.—Anaphase showing four omitted vesicles and one included in one of the chromosome groups.

Fig. 43.—Anaphase in face showing four vesicles.

Fig. 44.—Telophase showing omitted and included vesicles.

Fig. 45.—Telophase showing omitted and included vesicles.

Fig. 46.—Two daughter-nuclei of the first segmentations division showing small vesicles.

Fig. 47.—Individual vesicles. Each horizontal line shows the vesicles from a single nucleus. [Magnification oc. 4, objective  $\frac{1}{12}$ .] The lowest vesicle appears to show its origin from two elements.

Fig. 48.—Tripolar figure (prophase) showing seven vesicles.

Figs. 49 and 50.—Two aberrant nuclei from this series of eggs.



Fig. 51.—Metaphase condition of an egg of *E. acutus* which had been treated for half an hour with 50 c.c. sea-water + 7 c.c.  $2\frac{1}{2}$  M. NaCl. The spindle is abnormally narrow.

Figs. 52-61.—Mitotic figures of eggs of *E. acutus* treated with 50 c.c. sea-water + 15 c.c.  $2\frac{1}{2}$  M. NaCl for half an hour.

Fig. 52.—Chromosomes within nuclear membrane; no vesicles.

Fig. 53.—A nucleus in the same phase as fig. 52 but with vesicles. The large vesicle looks as though it might have been formed from more than one centre.

Fig. 54.—Nucleus in a slightly earlier stage than figs. 52 and 53, but with two well-formed vesicles.

Fig. 55.—A slightly later stage than fig. 53.

Figs. 56 and 57.—Prophases. Nearly all the chromosomes show evidence of giving rise to vesicles.

Fig. 58.—Prophase. The chromosomes are not distinguishable from one another, being very irregular in shape, and have given rise to small irregular masses of chromatin.

Fig. 59.—Slightly later stage than figs. 56 or 57. Five or six vesicles.

Fig. 60.—Similar mitotic phase showing seven vesicles.

Fig. 61.—Irregular anaphase with a very large vesicle which is very faintly stained and contains small granules at its periphery.

## PLATE 27.

### Figs. 62-71.

Fig. 62.—Chromosome group from a spindle of an egg of *E. esculentus*, Series C. All the chromosomes are abnormally swollen, and in the right-hand group is a large, irregular mass of chromatin. (Drawn under ocular 4, 2 mm. oil-immersion objective.)

Fig. 63.—The chromosomes of fig. 21 drawn under higher magnification. *Cent.*, centrosome.

Figs. 64-66.—Chromosomes from eggs of *E. acutus*. Series B.

Fig. 64.—Chromosome groups of an egg, showing thirty-four and thirty-five chromosomes at the two ends of the spindle, with three chromosomes and two vesicles omitted, thus making seventy-six elements in all. [Magnification oc. 4, objective  $\frac{1}{12}$ .]

Fig. 65.—Chromosome groups from a similar egg to the above, showing thirty-three chromosomes at each end and five omitted vesicles.

Fig. 66.—Anaphase groups containing thirty and thirty-one chromosomes respectively. No vesicles.

Figs. 67-69.—Chromosomes from eggs of *E. acutus*. Series C.

Fig. 67.—Anaphase with thirty-seven (?) and thirty-five (?) chromosomes. A double element is seen omitted at the equator of the spindle. Many of the other chromosomes seem to be paired.

Fig. 68.—Anaphase with four omitted chromosomes.

Fig. 69.—Early anaphase. Some chromosomes have only just divided and appear to be lagging behind the rest.

Figs. 70 and 71.—Chromosomes from eggs of *E. acutus*. Series C.

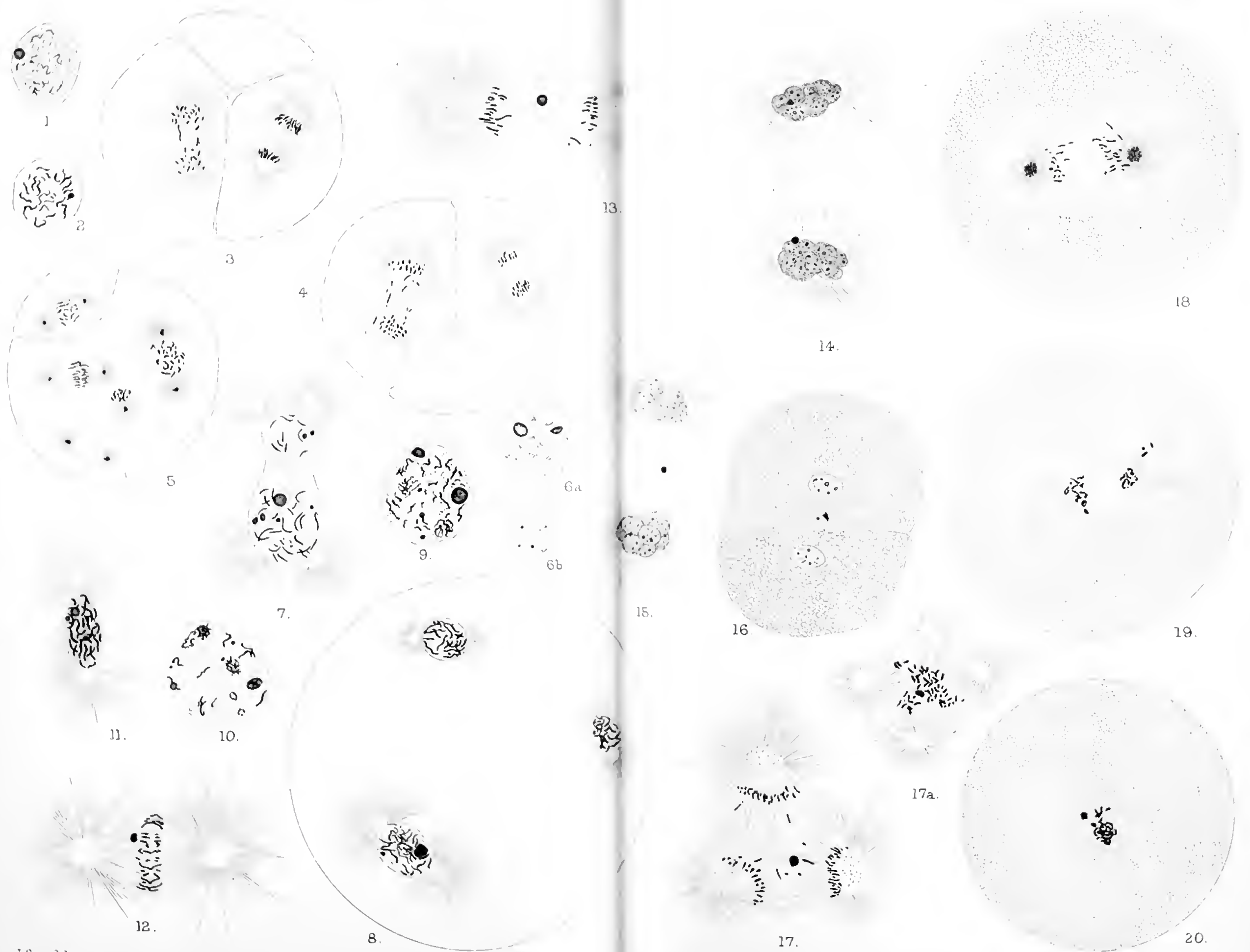
Fig. 70.—Equatorial plate with thirty-five chromosomes and three vesicles.

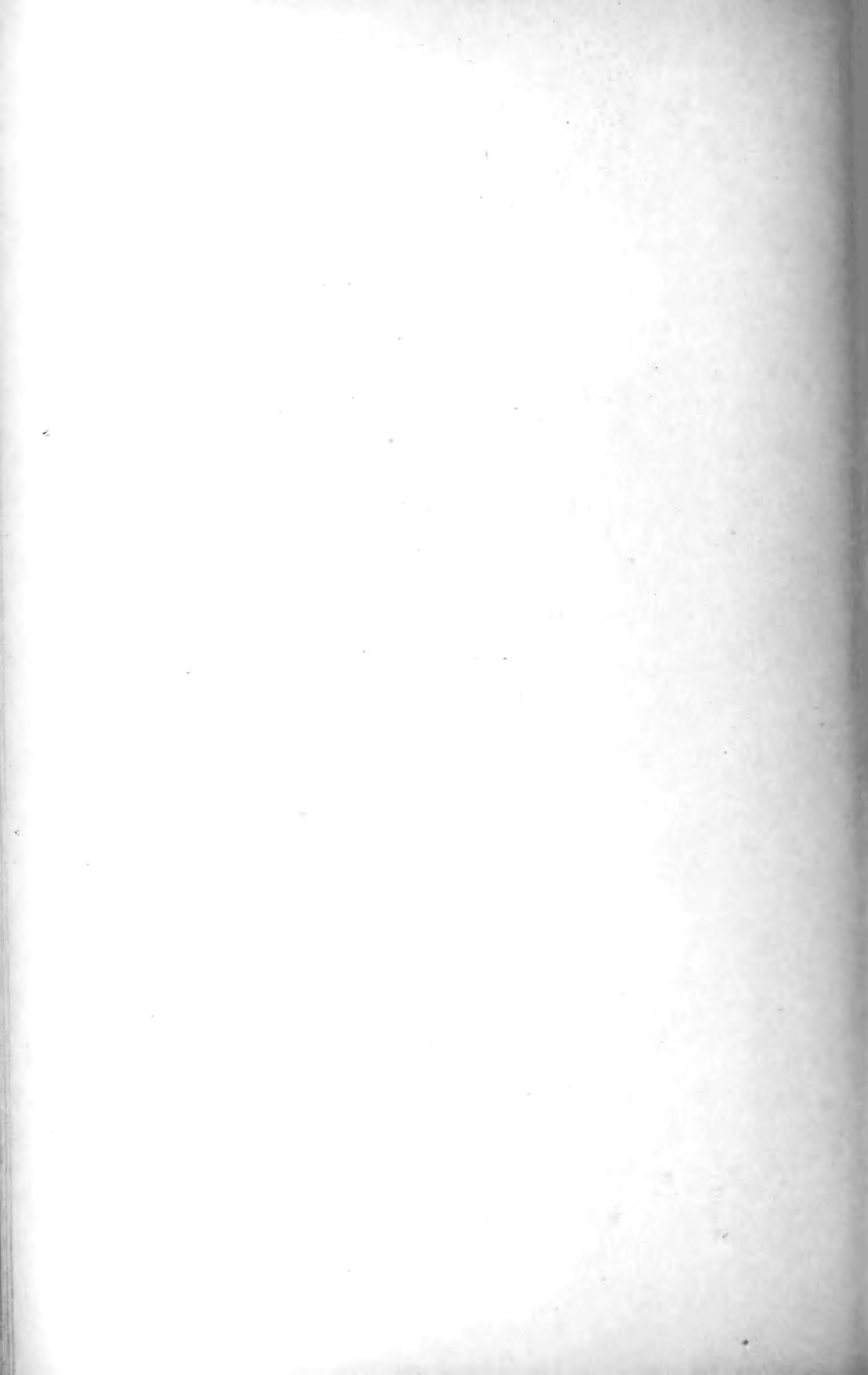
Fig. 71.—Prophase with thirty-four chromosomes and three stained vesicles. The large unstained vesicle is regarded as having been cut twice. Hence there is a total of four vesicles in this section.







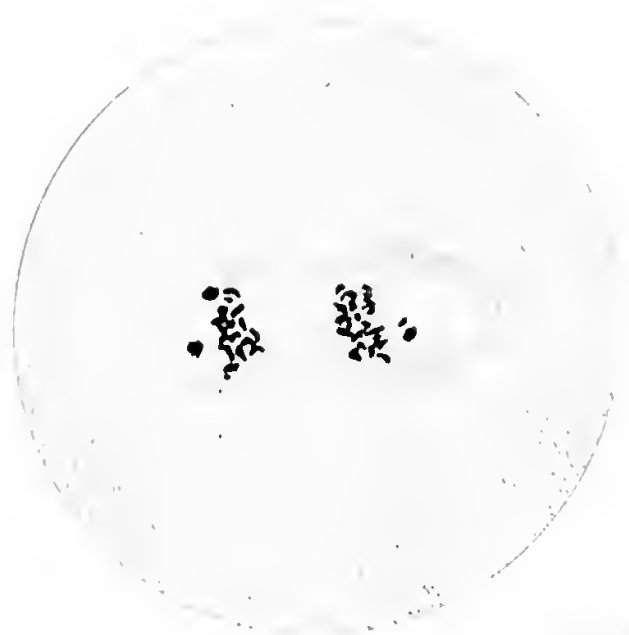








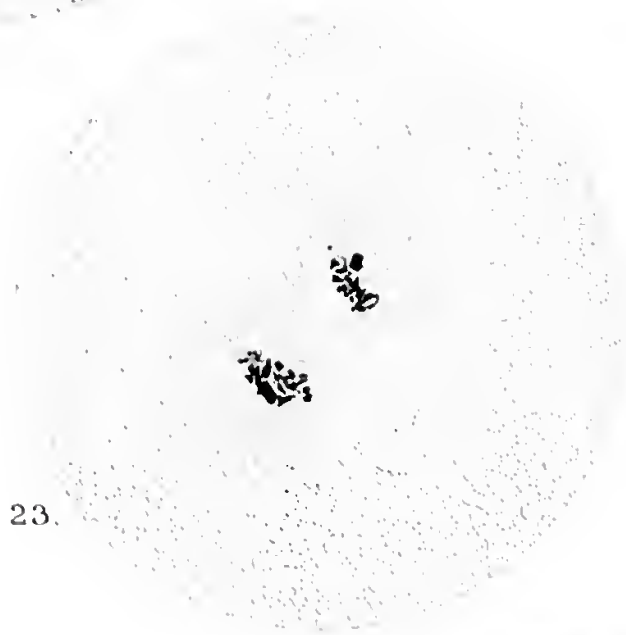




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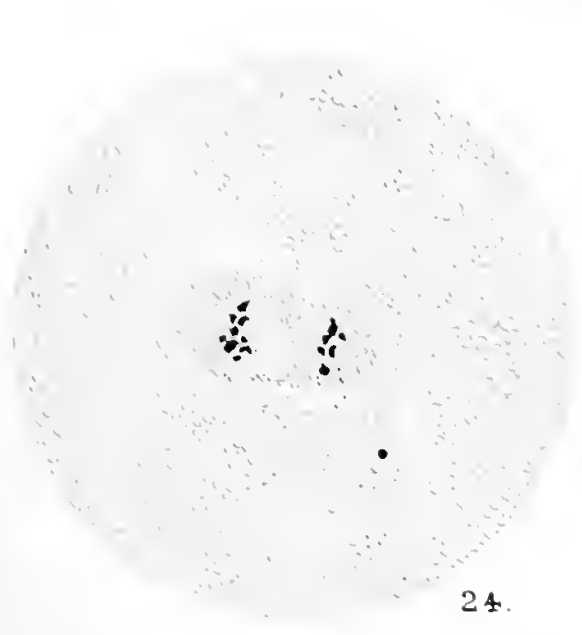
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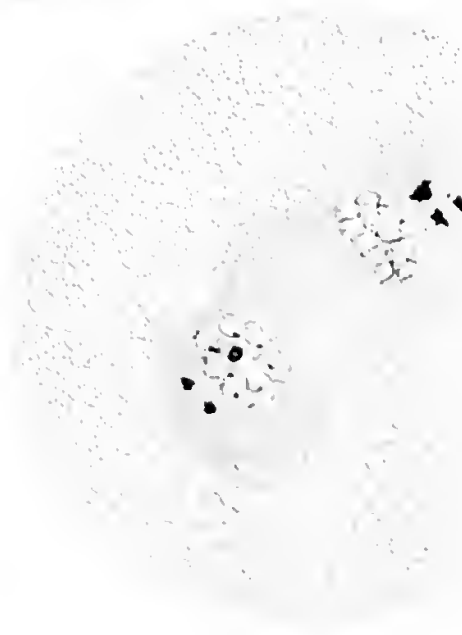
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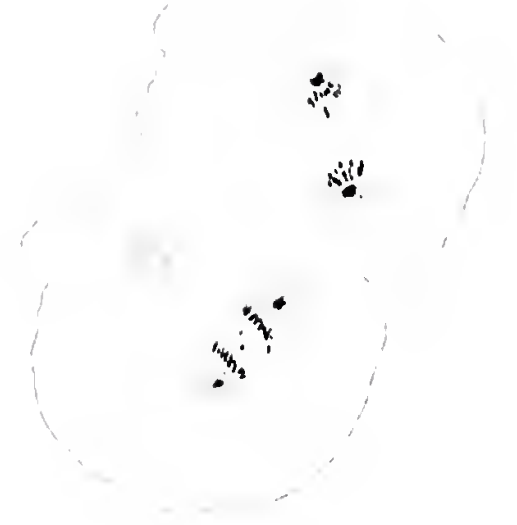
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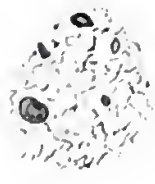








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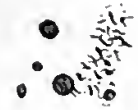
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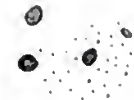
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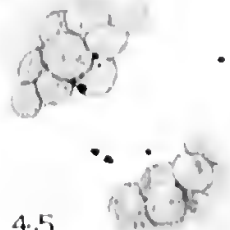
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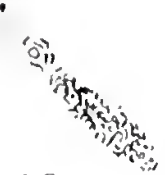
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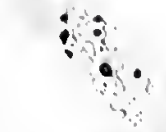
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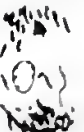
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62.



cent.

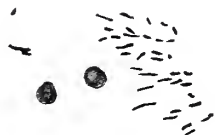


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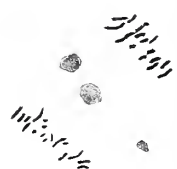
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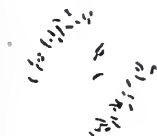
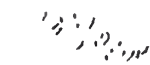
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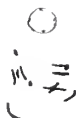
68.



69.



70



71.





## Cytological Observations on the Early Stages of Segmentation of *Echinus* Hybrids.

By

**L. Doncaster, M.A.,**

Fellow of King's College; and

**J. Gray, B.A.,**

Scholar of King's College, Cambridge.

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With Plates 28 and 29.

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### INTRODUCTION.

THE material on which the greater part of the observations here described was made was handed over to us by Messrs. Shearer, De Morgan and Fuchs, and was collected by them while carrying out experiments on the characters of the plutei in hybrids between the British species of *Echinus*. A preliminary account of their results has been published,<sup>1</sup> and their fuller paper appears concurrently with this. In view of the fact that in some of the hybrids the plutei, whichever way the cross was made, were essentially of the maternal type, it was clearly of interest to determine whether an elimination of paternal chromosomes takes place comparable with that described by Baltzer,<sup>2</sup> who has found that in the early stages of hybrids between different Echinoid genera, paternal chromosomes are eliminated in those cases in which the plutei are of the maternal type. The species used in the present experiments were *Echinus esculentus*, *E. acutus*, and *E. miliaris*, the three species of *Echinus* found at Plymouth, where the experiments were made. The material obtained

<sup>1</sup> 'Journ. Marine Biol. Soc.,' ix, 1911, p. 121.

<sup>2</sup> 'Arch. f. Zellforsch.,' v, 1910, p. 497.

in 1911 consisted of segmenting eggs of these three species, and of the hybrids *esculentus* ♀ × *acutus* ♂, *acutus* ♀ × *esculentus* ♂, and *acutus* ♀ × *miliaris* ♂, preserved at intervals of from half an hour to five hours after fertilisation.

In 1912, besides a few additional batches of the same crosses, we obtained eggs of *esculentus* ♀ × *miliaris* ♂ and *miliaris* ♀ × *esculentus* and *acutus* ♂. The great majority of the eggs sent to us were in stages from shortly before the conjugation of the pronuclei up to 2- or 4-cell stages; very few had reached the third segmentation division. Several samples of eggs were from batches the remainder of which were reared to plutei.

The preservatives used were sublimate-acetic, sublimate-nitric, Flemming's, Hermann's and Perenyi's fluids. The preservation varied in different cases; in general, sublimate-acetic and Flemming gave the best results. The eggs were sectioned (the sections we made ourselves were 5 or 7  $\mu$  in thickness), and stained on the slide with Heidenhain's iron-haematoxylin. In counting chromosomes we have found it absolutely necessary to draw every spindle; repeated attempts to count by eye have shown that by this method a number smaller than the true one is usually recorded.

Before describing the hybrid eggs, a few words are needed on those of the pure species. The greater part of the work has dealt with crosses between *acutus* and *esculentus*; of pure *miliaris* comparatively little material was available, and an account of it will be postponed until the *miliaris* hybrids are dealt with. Of *acutus* and *esculentus* we had a considerable supply of material from the early stages of fertilisation up to the second segmentation division. No account is needed of the stages of fertilisation nor of details of the segmentation mitoses except those relating to the chromosomes. We have determined the chromosome number in both species as 38; in early anaphase groups seen in face this number can frequently be counted with confidence (Pl. 28, figs. 1, 2, 3), and where a lower number is found it is

probable that some chromosomes are missing or concealed. The chromosome groups of the two species are very much alike; we have spent a considerable time in trying to discover points of difference which might be used in the study of hybrid eggs, but have failed to find any that are trustworthy. The chromosomes differ considerably in size and shape; in early anaphase figures seen in side view the following points are usually recognisable. (Fig. 3): Two chromosomes are noticeably longer than all the rest, and commonly complete their division slightly later than the remainder. Two are U- or V-shaped; if seen with the two limbs almost superposed they are recognisable by their apparently greater thickness. About four are longer than the remainder, though shorter than the two long ones mentioned above, and are not usually hooked at the ends; these, however, are not always distinguishable from the somewhat shorter rod-shaped chromosomes. The remainder are about equally divided between bodies the length of which is several times as great as their width, and shorter rods which often appear as oval or round dots if seen slightly obliquely. The chromosomes of both the last classes are frequently hooked at the end towards the pole; sometimes this is so pronounced as to cause them to resemble the V-shaped bodies mentioned above. Since the various classes described grade into one another almost imperceptibly, and are so similar in the two species, we have not been able to distinguish paternal and maternal chromosomes in the hybrid eggs in the crosses between *acutus* and *esculentus*.

The eggs of the pure species differ from those of the hybrids in the greater uniformity of the rate of development. In any batch of eggs of *acutus* or *esculentus* the majority are in stages of development not very far removed from one another, though there is not absolute uniformity. In the hybrid eggs, however, there is great diversity, and this appears to be the case to about the same extent whichever way the cross is made. In batches of which some eggs have reached the four-cell stage, others will show prophases of the

first division, or stages of conjugation. A considerable proportion of the eggs is also usually not fertilised.

#### THE HYBRIDS BETWEEN *ESCULENTUS* AND *ACUTUS*.

##### (i) *Esculentus* ♀ × *acutus* ♂.

In the eggs of this cross the behaviour of the nuclei appears to be perfectly normal. The conjugation of the pronuclei differs from that in *acutus* ♀ × *esculentus* ♂ described below in the fact that the sperm-nucleus often reaches a size nearly equal to that of the egg-nucleus before the two begin to unite, and that chromosomes are visible in both nuclei before conjugation. The mitotic figures are like those of the parent species, quite regular in every respect as far as the second segmentation division, and no trace could be found of chromosome elimination. The chromosome number in the first and second divisions appears to be 38 as in the parent species; elimination, if it occurs at all, does not take place until a later stage (Pl. 28, figs. 4, 5).

##### (ii) *Acutus* ♀ × *esculentus* ♂.

The mitotic figures in eggs from this cross differ conspicuously from those of the converse cross or the parent species, and it is to them that the greater part of our attention has been devoted. The conjugation of the nuclei appears to take place without serious abnormality. We have many examples of the two nuclei lying in contact, the sperm-nucleus distinguished by its smaller size and conspicuous central mass of chromatin. Rather later stages show that the sperm-nucleus becomes absorbed into the egg-nucleus before this chromatin mass breaks up, while the male nucleus is still quite small, and before the chromosomes of the egg-nucleus are formed; the rather elongate zygote nuclei with an aster at each end, such as are common in pure *acutus* eggs and in the cross, *esculentus* ♀ × *acutus* ♂, are not



usually found. In pure *esculentus* the process of conjugation appears to be more like what we have found in the *esculentus* ♂ hybrids. The zygote nucleus swells, irregular masses of chromatin appear within it, and become concentrated into small elongated chromosomes, the number of which is about thirty-eight. As the nuclear membrane disappears, and the spindle is completely formed, the chromosomes, at this stage scattered irregularly, become shortened and thickened, and often clumped in pairs or groups, so that a count usually gives a number much lower than that at rather earlier or later stages. Sometimes, however, they are scattered more separately, so that each can be seen distinctly, and here again thirty-eight may be counted (fig. 6, *a, b*). At about this stage, however, the abnormal feature of the mitosis appears; small vesicles of varying size and number appear among the chromosomes, distributed irregularly on the spindle (figs. 7-11). In eggs preserved with osmic fixatives these vesicles are faintly and evenly stained, except that the edge is more deeply stained than the centre; in sublimate preparations they have more the form of minute nuclei with small stained dots under their enclosing membrane, and are rather similar to the vesicles normally formed by the chromosomes in the late anaphase as they cluster round the pole of the spindle, although quite distinct from them.


A comparison of a series of prophase figures, or even of the vesicles in one figure, leaves no doubt that they are formed from chromosomes, for all stages may be found between a chromosome which appears somewhat swollen and a fully formed vesicle (figs. 7, 8, *a-z*). But it is also quite clear that in many cases the whole chromosome is not used up in forming the vesicle; often apparently normal chromosomes may be seen to which a vesicle is attached, either at its side or at one end. The number of vesicles which are formed at this stage varies from one or two up to about six or seven, or occasionally even as many as twelve, as shown in the counts in Table I. The considerable variation in the number of chromo-

somes and of vesicles is due in part to the fact that the vesicles are being formed at the stage at which the counts were made, and in some nuclei others might still have been produced; and as regards chromosomes, to the fact that at this stage they show a strong tendency to become clumped. The counts in Table I were made by eye; the number of vesicles in each case may be regarded as trustworthy, of chromosomes only approximate.

TABLE I.—Numbers of Chromosomes and Vesicles in Prophase Figures (first division), *acutus* ♀ & *esculentus* ♂.

Chromosomes.	Vesicles.	Chromosomes.	Vesicles.
31 .	12	29 .	6
29 .	9	35 .	2
32 .	6	32 .	2
31 .	2	29 .	3
33 .	3	31 .	0
28 .	1	32 .	3
33 .	1	32 .	4
31 .	6	32 .	7
28 .	2	31 .	3
31 .	3	32 .	3
		33 .	5

We at first believed that each vesicle was formed from a chromosome which was entirely used up in the process, but a detailed examination and comparison of different stages makes this view untenable. We believe that the vesicle formation may be explained as follows: In the late prophase stages, when the normal chromosomes are beginning to split longitudinally, some of the chromosomes, instead of splitting, tend to swell up and form vesicles as the normal chromosomes do in the late anaphase stage. Every gradation may be found between chromosomes which behave normally and those which are completely converted into vesicles.

Some merely become swollen and faintly stained in the centre; others develop a vesicle at one side or end while the rest of the chromosome appears normal; others, again, split more or less completely, but a vesicle is formed by one or possibly both of the longitudinal halves. Whether a chromosome of which one half forms a vesicle divides completely or remains undivided in anaphase, seems to depend to some extent on its position on the spindle at the time when the vesicle develops. Before the equatorial plate stage the chromosomes are scattered quite irregularly over the spindle, but already may show traces of the longitudinal split (fig. 9, *a*, *b*). If at this stage one half of a chromosome develops a vesicle, it prevents the whole chromosome from reaching the equatorial plate, with the result that both halves of the chromosome are included in the anaphase group belonging to the pole of the spindle to which the chromosome happened to be nearer in the prophase. This is especially well seen in the case of one chromosome which is conspicuously longer than the rest. Two such long chromosomes are found in both *esculentus* and *acutus*; the hybrid also has two, one doubtless derived from each parent. In the pure species these long chromosomes complete their division rather later than the smaller ones, making bracket-shaped figures, , just before the complete separation of the halves. In the hybrids it may sometimes be seen that one of them is in this stage in the middle plane of the spindle, while the second, half of it bearing a vesicle, lies between the equator and one of the poles (fig. 18*b*).

At the close of the prophase the normal chromosomes arrange themselves in an equatorial plate, and are at this stage rather long and bent. Some of them are already completely transformed into vesicles; to some, vesicles are attached, and probably others have already formed and thrown off a vesicle. The vesicles may be included in the equatorial plate, or remain scattered on the spindle; some are nearly always left in the equatorial plane just outside the spindle, and take no further part in the division. The chromosomes now divide along the split of which traces can

be seen in earlier stages, and the halves pass to the poles. We have already pointed out that in some, to which vesicles are attached, this division is incomplete and both halves probably pass to the same pole. There are occasional indications also that sometimes a chromosome which has not actually become vesicular, but which shows traces of swelling, may fail to divide, and pass entire to one pole. For this reason, in some early anaphases, while the two halves of most of the chromosomes are easily recognisable, some appear to be without mates, and the numbers in the two daughter-groups are not always equal. The numbers of chromosomes and vesicles found in anaphases of the first division are given in the counts recorded in Table II, which are all made by drawing. The numbers bracketed together indicate the two daughter-groups of one spindle; the total number of vesicles is given by the first figure, that of typical, unshrunk vesicles by the figure in round brackets ( ).

TABLE II.

Chromosomes.	Vesicles.	Chromosomes.	Vesicles.
32-35 } . .	9 (5)	36 } . .	5 or 6
33-35 } . .		30-32 } . .	
34-36 } . .	4	70-75† . .	1
35 } . .		30-32 } . .	2
31-34 } . .	5	32 } . .	
33-36 } . .		37 } . .	7
29* } . .	0	30-31 } . .	
30* } . .		37 } . .	5
		35 } . .	
		64-65† . .	5

\* Numbers approximate.

† Daughter-groups not distinct enough to count separately.

The exact number of vesicles in anaphase is difficult to determine, for while some of them appear to have increased in size, others appear to have shrunken and become reduced to

round, deeply stained bodies, which are not always easy to distinguish from large yolk-granules or sometimes from normal chromosomes. Every stage may be found between these stained dots and true vesicles, and in the telophase the normal chromosomes within the daughter-nuclei give rise to chromatic dots exactly similar in appearance. The fate of the vesicles depends upon their position on the spindle; those which lie among the chromosomes of the anaphase groups are carried with them to the poles and become included in the daughter-nuclei, while those which lie on the periphery of the spindle or just on its equator remain where they are, and are excluded from the nuclei. They may commonly be found along the line of the cell division in the 2-cell stage, already considerably shrunk and reduced to stained dots. The number eliminated as counted in late anaphase figures before the cell-division varies from none to about five, but in 2-cell stages a larger number of chromatic dots may sometimes be seen, and it is possible that they break up as they undergo degeneration. Figs. 10-14 illustrate anaphase and telophase stages of the first segmentation division.

Before proceeding to describe the second segmentation mitoses we must refer to a curious phenomenon which is very common in the first division figures. In a very large proportion of anaphases of the first division, and not rarely before the chromosomes have become arranged in the equatorial plate or even immediately after the dissolution of the nuclear membrane, it is seen that the centrospheres of the spindle have become divided, so that what at first appears as a bipolar or more commonly quadripolar spindle results. The extent of the division varies; the two halves of the sphere may be close together so that the spindle-fibres are not seriously deranged (figs. 10, 11), or they may, even in prophase, be widely separated, and a small secondary sheaf of spindle fibres may sometimes be seen between them (figs. 15, 16). At first we took these spindles with divided poles to be multipolar spindles caused by the entrance of more than one

spermatozoon, but an examination of a series shows that every stage occurs between normal spindles, through those with poles just divided to examples with four, or sometimes three quite distinct spheres to which the fibres converge.<sup>1</sup> A further difference is that in no case have we found a noticeably abnormal number of chromosomes; sometimes they appear to be considerably below the normal number, due doubtless to "clumping," but never conspicuously above the number expected. In one batch of eggs of pure *esculentus*, on the other hand, true tripolar and quadripolar spindles are of very frequent occurrence, in fact hardly any of the eggs are dividing normally; but in this case the chromosome number is constantly about either one and a half times or twice the normal (38). For example, in this batch of pure *esculentus* four counts of chromosomes on abnormal spindles (late prophase) gave respectively 52, 70, 71 and about 65 chromosomes (the numbers are only approximate); numbers of this kind have never occurred in the *acutus* ♀ × *esculentus* ♂ hybrids. We can only conclude, therefore, that in eggs of this cross there is a tendency for the centrospheres to divide prematurely, but the daughter-spheres rarely separate so widely as to cause an abnormal cell division. A moderate division of the spheres has been seen in some anaphase figures of pure *acutus* and of the converse cross *esculentus* ♀ × *acutus* ♂, but in these eggs it is never so extensive as in the cross *acutus* ♀ × *esculentus* ♂.

In the prophase of the second segmentation mitosis the chromosomes appear within the nucleus as elongated, more or less bent rods, like those of the same stage in the first division, but the smaller size of the nuclei and frequent interlacing of the chromosomes make trustworthy counting very difficult. Within the nucleus sometimes a varying number of faintly stained, round bodies may be seen, which we at first took for vesicles which had been included in the nucleus in the first telophase, but the inconstancy of their occurrence

<sup>1</sup> Boveri has figured similar divided poles in *Echinus microtuberculatus* ('Zellenstudien,' iv, Taf. v, fig. 59).

makes this interpretation very doubtful. When the nuclear membrane dissolves the figures are closely like those in the first prophase; the chromosomes become scattered over the spindle, and, as before, some of them are seen giving rise to vesicles (fig. 17, *a, b, c*). These differ from those of the first segmentation mitosis only in being in general somewhat smaller, and in the fact that complete chromosomes appear less often to become entirely vesicular; the vesicles seem rather to be formed on the ends or sides of the chromosomes, and often quickly become separated from them. The fact that the average number of vesicles in the second division is about the same as that in the first, and that the chromosome number, as counted in anaphase, shows no further diminution, suggests that the same chromosomes which produced vesicles in the first mitosis again do so in the second.

The division of the poles of the spindle is less conspicuous in the second segmentation mitosis; in anaphase they are often elongated transversely to the axis of the spindle, or divided to a small extent, but exactly similar figures are not uncommon in pure *esculentus* eggs in the same stage. The vesicles, as before, are scattered among or outside the chromosomes, and in anaphase some are included among the daughter groups and enter the nucleus of the 4-cell stage; others remain in the centre or round the edges of the spindle and are eliminated (figs. 18, 19, 20).

Counts of late prophase and anaphase groups gave the results recorded in Table III; the prophase figures were counted by eye, the anaphases by drawing. As in the case of the first division the anaphase figures are more trustworthy. Brackets on the right side of the numbers indicate anaphase groups of the same spindle; on the left side spindles of the same egg.



TABLE III.—Counts of Chromosomes and Vesicles in Second Divisions.

PROPHASES.		ANAPHASES.			
Chromo- somes.	Vesicles.	Chromo- somes.	Vesicles.	Chromo- somes.	Vesicles.
{ 33	. 6	34-35	4	{ 37	5
{ 35	. 3	35		{ 37	
35	. 6	35	7	{ 35*	. 6
{ 28	. 6	35		{ 35	7
{ 29	. 8	{ 35	6	{ 36	
32	. 2	{ 35		{ 38	6
31	. 1	{ 35	6	{ 35	
{ 32	. 4	{ 36		{ 33-35	5
{ 32	. 3	{ 33	6	{ 33-34	
{ 30	. 5	{ 35		{ 34-35	4
{ 32	. 3	{ 35	5	{ 34	
{ 30	. 3	{ 35		{ 71-72†	. 5
{ 31	. 5	36	8	{ 31-34	6
		35		{ 33	
		35-36	5	{ 31	5
		33-34		{ 34	
				{ About 30*	1

\* In these figures the chromosomes had not yet divided.

† Two ends of spindle not accurately separable: two or three chromosomes possibly cut in two sections.

It will be seen that while there is considerable variation in the chromosome number, the numbers in the two spindles in most eggs are in fair agreement, and further, that the number of vesicles added to the chromosome number is often considerably above 38. This, and the fact that there is no diminution in the chromosome number as compared with that in first division anaphases, confirms us in our belief that in the second division the vesicles are given off from chromosomes rather than that whole chromosomes are converted into vesicles. Occasionally also in second division anaphases, chromosomes which have clearly divided, and are travelling

in a normal way to the poles, are seen to have vesicles attached to them. We conclude, therefore, that the tendency to vesicle-formation is diminished in the second division, and that nearly all the chromosomes which entered the nucleus of the 2-cell stage are able to divide and move to the poles in the normal way. Our material does not provide any examples of segmentation divisions later than the second in the cross *acutus* ♀ × *esculentus* ♂, but we defer any discussion of the facts observed until the hybrids with *E. miliaris* have been described.

#### *E. miliaris* AND ITS CROSSES WITH *ACUTUS* AND *ESCULENTUS*.

The material obtained in 1911 included only one cross with *E. miliaris*, viz. *acutus* ♀ × *miliaris* ♂. Unfortunately the supply of pure *miliaris* eggs was small and not very good, so that we cannot give so full an account of it as of the other species. All the eggs showing mitotic spindles in pure *miliaris* were in the 4-cell stage or later, and owing to the small size and crowded state of the figures counting was difficult. The chromosome number appears to be smaller than in the other species; many counts, especially of equatorial plates, gave numbers ranging from 30 to 32 or 33, but in two anaphase groups in one spindle, in which the chromosomes were very clearly shown in face, 34 may be counted at each pole (fig. 21), and this number is confirmed by counts of the cross *esculentus* ♀ × *miliaris* ♂ (1912). The chromosomes are more nearly alike in size than in *esculentus* and *acutus*.

#### *Acutus* ♀ × *miliaris* ♂.

In the eggs from the cross *acutus* ♀ × *miliaris* ♂ the spindles differ noticeably from those of the *acutus* and *esculentus* crosses (figs. 22-25). The spheres with their radiations are very large and conspicuous, and only rarely show any division. The spindles are much narrower, with the

result that the chromosomes are more crowded; this relative narrowness may be due partly to the absence of divided poles, which in the *esculentus* hybrids cause considerable widening of the whole figure, but this cannot completely account for the difference, for in the exceptional cases of divided poles in the *miliaris* cross, the spindles are still very narrow. The general appearance of the spindles and asters is much more like that of pure *miliaris* than of *acutus*, suggesting that the centrosome introduced by the *miliaris* spermatozoon can cause mitotic figures of the *miliaris* type to develop in *acutus* eggs. Our observations on the hybrid eggs have been made almost entirely on two lots, which gave in some respects dissimilar results. One lot consists of stages from the beginning of the first to the beginning of the second segmentation division, all the stages of the first division being well represented. The second lot contains much more advanced eggs, up to the 16-cell stage at least, but also includes early stages from prophases of the first segmentation division onwards. In a preliminary examination of the first lot we failed to find any trace of elimination or of vesicle formation; both equatorial plates and anaphases were somewhat irregular, but the conspicuous vesicles found in the *acutus* ♀ × *esculentus* ♂ cross appeared quite absent. In the second lot, however, vesicles were not rarely found in the earlier divisions, and when present were of the same kind and as conspicuous as in the cross with *esculentus* ♂. A re-examination of the second lot showed that vesicles were sometimes present, but were in most cases very small, and attached to chromosomes as in prophases of the *acutus* ♀ × *esculentus* ♂ cross (fig. 22). Further, in metaphase and early anaphase it is sometimes, though not by any means always, possible to see that a few (one to three) chromosomes are swollen, irregular in shape, and without any trace of a division at a time when the halves of the remainder are beginning to separate. In later anaphases the majority of the chromosomes are rod-shaped, long and narrow, but sometimes a few are much larger and more ovoid in shape. In anaphases occasionally a minute vesicle may be seen left on the spindle,

but these are always so small and faint as to be recognisable with difficulty, and in telophase and the 2-cell stage no trace of elimination is to be found.

In the second lot of eggs typical though small vesicles are commonly to be found in the first division, and may be seen, though with progressively less frequency, in the second, third and sometimes the fourth. The vesicles in these eggs are in most cases still attached to chromosomes, and are included with them in the daughter-plates, and appear to be carried into the nucleus. Vesicles are rarely if ever left on or outside the spindle as in the first and second divisions of the cross with *esculentus*. The interpretation of these eggs is made difficult by the fact that those in the earlier stages are almost all abnormal; many have multipolar spindles and nuclei dividing without division of the cytoplasm, and frequently there is evidence of polyspermy. The later stages of this batch, however, from the 8-cell stage onwards, appear to be normal, and an occasional vesicle may be found in them also. We suggest that the behaviour of the eggs in the two lots may be reconciled as follows: In the first divisions of the cross *acutus* ♀ × *miliaris* ♂ a small but varying number of chromosomes may show a tendency to form vesicles as in *acutus* ♀ × *esculentus* ♂; in normally developing eggs the vesicles are usually small and do not become detached from the chromosomes, but when the tendency to form vesicles is present the chromosomes affected probably fail to divide and are carried entire to one or other pole. In eggs the development of which is delayed and which are abnormal through polyspermy or other causes, larger and more conspicuous vesicles are formed, which, however usually remain attached to the chromosomes and so are not eliminated from the daughter-nuclei. The non-division of some of the chromosomes is not an easy matter to prove, and in many eggs all appear to behave quite normally, but that in some eggs one or more fail to divide is suggested by three facts: (1) The great difference in thickness among the chromosomes in some anaphases suggests that some of them have failed to divide.

(2) In early anaphase it is often possible to see which chromosomes in the daughter-groups correspond with each other, and it is usually the swollen and irregular ones which appear to be without mates; unfortunately, however, the narrow and crowded spindles make the determination of this point uncertain in many cases. (3) The third piece of evidence is from counts of the chromosome number. If *acutus* has 38 and *miliaris* 34 chromosomes, the hybrid should have 36. We have found two eggs in which the spindle is replaced by a monaster (fig. 26), and in these the chromosomes, though some of them are constricted, have not divided, and in each 36 can be counted, in one case with confidence, in the other with great probability.<sup>1</sup> Prophases after the nuclear membrane is dissolved are in this cross usually too crowded for counting, but we have found one in which about 36 are also present. In almost all other counts of both batches of eggs

TABLE IV.—Chromosome Counts in *Acutus* ♀ × *miliaris* ♂.

Prophases and Metaphases.	Anaphases.
36 (39 ?) (monaster)	36 }
36 + 1 small vesicle (monaster)	34 }
32 (35 ?) + 1 vesicle }	35
36 + 1 „ }	35
31* + 1 „ }	35 (33-36)
29* + 1 „ (?) }	35 }
34 }	32 (33 ?) }
37 }	34 }
30-34 + 1 vesicle }	35 }
34-35 }	35-37 }
	33-55 }
	31 }
	37 }

\* These numbers are probably too low.

<sup>1</sup> That the number when no vesicles are present should be 36 is further indicated by the similar cross *esculentus* ♀ × *miliaris* ♂ (see below), in which the number is almost certainly 36.

the number is lower, as is shown in Table IV. The two ends of one spindle are bracketed as before.

The small size of the chromosome groups, with the resultant crowding, doubtless makes the numbers in Table IV lower than the true figure, and further, when the chromosomes are in very close proximity, it is always possible for two of them, if the fixation is not very perfect, to become so united that they appear as one of double thickness. In the pure *miliaris* eggs, although we have sections in which 34 chromosomes can be counted with little doubt, the majority of counts gave from 31 to 33, and hence we are not disposed to lay very great weight on the counts in the *miliaris* hybrids. Nevertheless the frequency with which the counts fall below the theoretical number 36, coupled with the facts that vesicle-formation on a small scale is not infrequently seen in the early stages of division, and that in early anaphases swollen chromosomes without visible mates at the other pole are also sometimes found, suggests that in some eggs at least a reduction in chromosome number may be brought about by non-division of one or more chromosomes.<sup>1</sup>

It appears, therefore, that in the *acutus* ♀ × *miliaris* ♂ cross a small number of chromosomes show a varying tendency to swell and form vesicles in the early stages of the first division; when the tendency is pronounced this may cause failure to divide in the metaphase, and they become carried to one or other pole. Exceedingly small vesicles are sometimes eliminated, but this process is not frequent and conspicuous as it is in the cross *acutus* ♀ × *esculentus* ♂. In the later divisions the abnormal tendency is less marked, and by the third or fourth segmentation

<sup>1</sup> A somewhat cursory examination of three batches of eggs of *acutus* ♀ × *miliaris* ♂, obtained shortly before going to press, confirms our account of this hybrid. The eggs, almost all of which are fertilised, are chiefly in the later stages of the first division. The spindles are like those obtained in 1911; most of the figures are normal, but a few show very small vesicles, and one case was found of a large vesicle eliminated in the telophase.

division all the chromosomes have usually regained their normal behaviour. When widely different numbers are counted at the two poles of one spindle in the later segmentation divisions (cf. fig. 27 *a, b*), it is possible that some chromosomes are still behaving abnormally, but another possible explanation is that owing to the irregularity of the equatorial plate the two halves of the chromosomes are still seen in one daughter-plate.

Comparing these conclusions with what has been described in the cross *acutus* ♀ × *esculentus* ♂, it is seen that in the latter cross a similar tendency is more strongly shown; some chromosomes swell up entirely to vesicles which are left out of the spindle, and others produce smaller vesicles, and in some cases probably fail to divide. The vesicles which are left near the equator of the spindle are eliminated. In the second division there is already evidence that the chromosomes are beginning to recover, and the results obtained from the cross *acutus* ♀ × *miliaris* ♂ suggest that in the later segmentation divisions their behaviour would again be normal. Owing, however, partly to the elimination of some entire chromosomes in the first division, and to the failure of others to divide, the chromosome number of the *acutus* ♀ × *esculentus* ♂ hybrid would be reduced from 38 to somewhere between 31 and 37, the number varying in different embryos, and probably sometimes in different cells of the same embryo.

The preceding account deals with the hybrid eggs obtained in 1911. In that year, owing to an oversight, no eggs of suitable age were obtained from the crosses *esculentus* ♀ × *miliaris* ♂, and of *miliaris* ♀ × *esculentus* and *acutus* ♂. These hybrids were obtained in 1912, but, as Shearer, De Morgan and Fuchs have recorded,<sup>1</sup> the plutei obtained from them differ considerably from those reared in 1911, and the *miliaris* eggs especially appear in this year to develop much less satisfactorily than in 1911. It cannot,

<sup>1</sup> 'Nature,' June 27th, 1912.



therefore, be concluded with complete confidence that our results obtained from these hybrids are entirely typical.

*Esculentus* ♀ × *Miliaris* ♂.

The eggs of this cross preserved in 1912 were mostly in the 2-cell stage, but a considerable number also show stages in the first division. Almost all are fertilised and in general are developing quite normally. No elimination of chromosomes nor formation of vesicles was observed, and the mitotic figures are in most cases as regular as in the eggs of the pure species. The spindles are less noticeably narrow than those described in the cross *acutus* ♀ × *miliaris* ♂, but are probably narrower than in pure *esculentus* eggs or in *esculentus* ♀ × *acutus* ♂; divided poles are not found. Counts of chromosomes leave little doubt that the number is 36. Seventeen counts of anaphase groups, in 11 eggs, gave 36 in nine cases (7 eggs), including nearly all the most satisfactory figures; 37 in two cases in which a pair, counted as two, are probably really one V-shaped chromosome; and in six counts either 34 or 35. Some of the cases in which 36 are seen are so clear that there can be little doubt that it is the true number (fig. 28). If our estimate of 34 for *miliaris* and 38 for *esculentus* be correct, 36 is the number to be expected. One V-shaped chromosome is commonly visible; there are two in *esculentus*, and none (probably) in *miliaris*. In a considerable number of the anaphase figures (about eight out of twenty examined on this point), no V could be found, and for a time we thought that this body might be comparable with the hook-shaped chromosome found by Baltzer in half the eggs of *Strongylocentrotus* and the horse-shoe found in half those of *Echinus microtuberculatus*.<sup>1</sup> Further study, however, has convinced us that in our material it is almost impossible to determine with certainty the presence or absence of this element; when seen sideways it is practically indistinguish-

<sup>1</sup> 'Arch. f. Zellforsch.,' ii, 1909, p. 549.

able from one of the thicker rods, and it may be present in one cell of a 2-cell stage, and apparently absent in the other.

Miliaris ♀ × Esculentus ♂ and Miliaris ♀ ×  
Acutus ♂.

These two crosses, the converse of the last two described, may be taken together. As has been mentioned above, the miliaris eggs in 1912 were very unsatisfactory, only a small proportion yielding larvæ even when fertilised by their own sperm. In our material of the hybrid eggs, in both cases an exceedingly small proportion showed evidence of fertilisation. In the cross with esculentus ♂ about 2 per cent. are beginning to develop; in that with acutus ♂ the percentage is considerably lower. Most of the developing eggs in both cases are in the 2-cell stage, but nearly all stages of both first and second segmentation divisions have been found, and at least one clear case of the conjugation of the egg and sperm nuclei.

The figures in both crosses are distinguished from those of the converse crosses by the greater irregularity of the chromosomes on the spindle. The extent of this irregularity varies; some figures are almost normal, in others the chromosomes are much scattered, but cases are rarely found in which an accurate count of chromosomes is possible. In one unusually regular first anaphase of miliaris ♀ × acutus ♂ cut transversely to the spindle, 35 may be counted at one end and about 36 at the other; in this case, however, four chromosomes are scattered entirely outside the main group around the pole. In late anaphases and telophases of both crosses it may often, but not always, be seen that one, two, or probably sometimes more, chromosomes lag to such an extent that they are not included in the daughter-nuclei (figs. 29, 30). In some cases they appear not to divide; in others the halves separate so late that they are left behind in the cytoplasm. Sometimes this causes the daughter-nuclei to have a tail-like

projection extending towards the line of cell-division; in others the daughter-nuclei are normal, but a stained mass of chromatin remains in the cytoplasm on the line dividing the two cells. In 2-cell stages such a chromatic mass, usually very small, but of varying size, is very commonly found pressed against the faces by which the cells are in contact, sometimes in only one of the daughter-cells, at others in both. In other eggs no sign of elimination is found, but it is possible that in some at least of these the eliminated chromosome has been absorbed.

The second division figures in our material are mostly in metaphase or early anaphase, so that it is difficult to determine with certainty whether any chromosomes are eliminated in them; in one case in an anaphase one chromosome appears not to be dividing (*miliaris* ♀ × *acutus* ♂), but in the few other cases observed no evidence of elimination in the second segmentation division was found. The spindles in these crosses, especially in the second division, are very short, with poles near together; not very infrequently nuclear division occurs not followed by cell-division.

In general, then, it may be concluded with regard to the *miliaris* crosses, that in (1) *acutus* ♀ × *miliaris* ♂ a small but variable number of vesicles, such as we have described in *acutus* ♀ × *esculentus* ♂, is sometimes formed, and that the chromosome number is possibly reduced thereby to a small extent. (2) In *esculentus* ♀ × *miliaris* ♂ there is no abnormality, and the observed chromosome number is that which is to be expected from our determination of the numbers in the parent species. (3) In the converse crosses *miliaris* ♀ × *acutus* and *esculentus* ♂, no vesicles are produced, but in the anaphase of the first division one or more chromosomes commonly either fail to divide, or divide so late that they are not included in the daughter-nuclei.

## SUMMARY AND DISCUSSION.

Our work was undertaken primarily in order to discover whether in the crosses described there was any systematic elimination of chromosomes, such as have been found by Baltzer in crosses between *Sphærechinus* and *Strongylocentrotus*, which might be correlated with the facts described by Shearer, De Morgan and Fuchs in their work on the hybrid plutei. Baltzer found that in the cross *Sphærechinus* ♀ × *Strongylocentrotus* ♂ no elimination of chromosomes occurs, and the hybrid plutei are intermediate between the parental types. In the converse cross (which is successful in a very small percentage of cases), sixteen chromosomes, regarded as belonging to the male (*Sphærechinus*) parent are eliminated, and the hybrid plutei, when exceptionally they can be reared, are of the maternal type. In our cases, Shearer, De Morgan and Fuchs have found, in the years preceding 1912, that in crosses between *esculentus* or *acutus* and *miliaris* the plutei, in certain characters at least, were of the maternal type, whichever way the cross was made. It seemed possible, therefore, that we might find elimination of chromosomes in the manner described by Baltzer.

For several reasons the work described above has followed somewhat different lines from those expected at the beginning. In 1911, the only cross with *miliaris* which was obtained before the season was too far advanced was *acutus* ♀ × *miliaris* ♂. In the hybrid eggs we found that some of the chromosomes developed vesicles, but no other elimination occurred except the possible non-division of certain chromosomes, about which we are uncertain. The vesicle formation, which appears to a small extent in eggs of *acutus* ♀ × *miliaris* ♂, is much more clearly shown in the cross *acutus* ♀ × *esculentus* ♂, species in which the characters of the plutei are so similar that the hybrid cannot be distinguished with certainty from the pure forms. We have therefore been

led chiefly to study the vesicle formation in this cross, on which a few words will be added below.

In 1912 we have obtained the remaining crosses with *miliaris*. Of these, we find that *esculentus* ♀ × *miliaris* ♂ behaves normally, with no elimination; *miliaris* ♀ × either *esculentus* or *acutus* ♂ shows only a small percentage of developing eggs, of which a considerable proportion at least show elimination of one, or at most a few chromosomes. We do not feel able to say with confidence whether these are paternal or maternal; in the few examples which are at the right stage of division, the length of the eliminated chromosomes suggests that they are paternal. We cannot, however, correlate this result with any confidence with the observation that the advanced plutei show maternal characters, for as Shearer, De Morgan and Fuchs have recorded, in 1912 the crosses with *miliaris* ♀ have shown a different behaviour from that of previous years, and have been as a rule of the paternal rather than of the maternal type. It is not certain, therefore, that our results obtained in 1912 are similar to what would have been obtained in 1911 or previous years. It is worthy of note, however, that in the cross *esculentus* ♀ × *miliaris* ♂, which in 1912, as in other years, has given purely maternal plutei, no chromosome elimination of any kind has been found.

With regard to vesicle-formation little need be said here, for the matter is thoroughly discussed in a paper by one of us published concurrently with this. We assumed at first that the chromosomes which develop vesicles are paternal, and that vesicle-formation is to some extent comparable with the elimination of paternal chromosomes described by Baltzer. Experiment with hypertonic solutions on eggs of the pure species, however, has made it very probable that the vesicles in the eggs of the cross *acutus* ♀ × *esculentus* ♂ are derived from the *acutus* (maternal) chromosomes, due probably to an alteration of the permeability or osmotic condition of the egg consequent upon the development within it of a foreign spermatozoon. Baltzer gives evidence on several

distinct lines that the chromosomes eliminated in his experiments were all paternal, so that if he is correct, it is probable that the vesicle-formation in our case is quite a different phenomenon. Tennent,<sup>1</sup> on the other hand, finds that both paternal and maternal chromosomes are eliminated in the cross *Arbacia* ♀ by *Toxopneustes* ♂, so that it is possible that elimination is not due simply to an incompatibility between the chromosomes of one species and the egg-cytoplasm of the other, but that, as suggested above, the physical condition of the cytoplasm is altered by the development within it of a foreign sperm-nucleus, causing the non-division or other abnormal behaviour of certain chromosomes, of either one or both species in different cases.

In any case a point of importance is that all these experiments give evidence of a physiological differentiation among the chromosomes. Some behave normally, others form vesicles, or fail to divide, and from the comparative constancy of the numbers it may be inferred that it is the same chromosomes in each case which are affected. We do not feel confident in identifying the chromosomes which form vesicles in the *acutus* ♀ × *esculentus* ♂ cross, but our general impression is that some at least of the affected chromosomes are the same in each case, suggesting that they differ in physiological characters as they do in shape and size. The physical cause of the vesicle-formation has been discussed by one of us elsewhere, but we put forward one additional suggestion which may possibly be worthy of consideration. Vesicles are produced in *acutus* chromosomes when the eggs have been treated with hypertonic solutions and have been returned to normal sea-water. It seems possible that the hypertonic solution withdraws water from the egg, causing it to have a higher concentration, both in the cytoplasm and nucleus, than the normal. At about the time the chromosomes are forming the egg is returned to water of lower concentration; the cytoplasm will then absorb water, and its concentration will thus be lower than that of the

<sup>1</sup> 'Journ. Exp. Zool.' xii, 1912, p. 397.



chromosomes lying in it. If, then, each chromosome can be regarded as a closed semipermeable membrane containing substances at a higher concentration than the surrounding cytoplasm now possesses, the chromosomes will absorb water, and in some cases swell so much as to produce vesicles.

In the cross *acutus* ♀ × *esculentus* ♂ the vesicle formation could be explained on the same hypothesis, if the *esculentus* spermatozoon takes up more fluid from the cytoplasm and especially from the egg-nucleus in forming the male nucleus than does the *acutus* sperm. It will act like the hypertonic solution in withdrawing water from the rest of the egg. The egg-cytoplasm will replace this water from the surrounding sea-water, but the chromosomes which have formed meanwhile in the egg-nucleus will have a higher concentration than the normal, and, as in the case of the eggs treated with hypertonic solutions, will show a tendency to swell and form vesicles. That the chromosomes may be regarded as being each enclosed in a semipermeable membrane is suggested by their normal behaviour in the late anaphase, when each chromosome swells up to form a vesicle in appearance like a small nucleus. Evidence in the same direction has been recently brought forward by A. A. Lawson<sup>1</sup>, though we doubt whether his conclusions are entirely applicable to our cases. The suggestion that in the cross *acutus* ♀ × *esculentus* ♂, the *esculentus* sperm acts like a hypertonic solution in withdrawing water from the egg-nucleus is supported by the fact, referred to in our account of the conjugation of the nuclei, that in this cross the sperm-nucleus conjugates with the egg-nucleus while it is still quite small and contains a compact mass of chromatin, and while the chromosomes of the egg-nucleus are not yet definitely visible. In the converse cross, on the other hand, in which no vesicles are formed, the sperm-nucleus becomes nearly as large as the egg-nucleus before the two come into contact, so that conjugation does not

<sup>1</sup> Lawson, "Nuclear Osmosis as a Factor in Mitosis," 'Trans. Roy. Soc. Edin.' vol. xlviii. pl. i, 1912, p. 137.



take place until both nuclei are fully formed and contain visible chromosomes. It is doubtful, however, whether this can be the complete explanation of the vesicle-formation, for without further qualification it does not provide a reason for the production of vesicles in the second segmentation division. That the vesicle-formation is a phenomenon depending on the disturbance of the osmotic relations between the chromosomes and cytoplasm seems nevertheless a hypothesis which should be taken into account.

### EXPLANATION OF PLATES 28 AND 29,

Illustrating Mr. L. Doncaster and Mr. J. Gray's "Cytological Observations on the Early Stages of Segmentation of *Echinus* hybrids."

[The figures were drawn with a Zeiss apochromat, 3 mm., n.a. 1.40 and with compens. o.c. 12. They are not all drawn to the same scale, figs. 5, 13, 14, 20, 22, 23, 29, 30 being on a smaller scale, figs. 8 and 17 on a larger scale than the rest. Since the chromosomes on the spindles are at different levels in the section, it was found that drawing by eye was preferable to using a camera. In some cases where the cell outlines are drawn, the nuclei or spindles are enlarged relatively to the cell.]

#### PLATE 28.

Fig. 1, *a, b*.—*E. acutus* ♀ × ♂. Two anaphase groups in face, from different eggs, 38 chromosomes in each. The two chromosomes outside the group to the left in 1 *b* belong to the other end of the spindle.

Fig. 2, *a, b*.—*E. esculentus* ♀ × ♂. Two anaphase groups in face, from the same spindle. Thirty-eight chromosomes in 1 *b*. 37 or 38 in 1 *a*.

Fig. 3, *a, b*.—*E. esculentus* ♀ × ♂. Anaphase (second division); spindle seen sideways. 3 *a* and 3 *b* are from successive sections; 38 at each end.

Fig. 4.—*E. esculentus* ♀ × *acutus* ♂. Anaphase, second division, in face; 38 chromosomes.

Fig. 5.—*E. esculentus* ♀ × *acutus* ♂. Anaphase, second division, side view.

Figs. 6-20.—*Acutus* ♀ × *esculentus* ♂.

Fig. 6, *a*, *b*.—*Acutus* ♀ × *esculentus* ♂. Two sections of prophase; 38 chromosomes, no vesicles at this stage.

Fig. 7.—Later prophase; formation of vesicles. The line round the spindle in figs. 6 and 7 represents the edge of the clear area in which the spindle lies.

Fig. 8, *a*-*z*.—Various shapes of chromosomes during vesicle formation. Early stages, first segmentation division.

Fig. 9, *a*, *b*.—Two sections of a late prophase, showing chromosomes beginning to divide and vesicles.

Fig. 10, *a*, *b*.—Anaphase, first division, showing vesicles left on equator of spindle, and divided poles. Some chromosomes added in both *a* and *b* from the next sections.

Fig. 11, *a*, *b*.—Later anaphase—only two vesicles. Five chromosomes added in 11 *a*, from the next section.

Fig. 12.—Telophase, showing normal chromosomes becoming vesicular at the poles; one large vesicle included among them; seven eliminated. Combined from two sections.

## PLATE 29.

Fig. 13.—Beginning of cell-division. Several vesicles eliminated. Combined from two sections.

Fig. 14.—Completion of cell-division. Several vesicles eliminated.

Fig. 15, *a*, *b*.—Late prophase showing considerable division of poles.

Fig. 16, *a*, *b*.—Extreme case of division of poles giving quadripolar spindle. Not all the chromosomes are shown; they were not more than 38.

Fig. 17, *a*, *b*, *c*.—Vesicle-formation in second division.

Fig. 18 *a*, *b*.—Early anaphase, second division; two sections of one spindle. There were also one small chromosome and three small vesicles in the next section.

Fig. 19, *a*, *b*.—Anaphase, second division. Two sections of one spindle. The small vesicles on the lower edge of 19 *b* were added from the next section. In 19 *a* one long chromosome is displaced by the razor.

Fig. 20.—Telophase, second division. Three vesicles eliminated on each spindle.

Fig. 21, *a*, *b*.—*E. miliaris* ♀ × ♂. Two anaphase groups in face, from one spindle: 34 chromosomes at each end.

Figs. 22, 23.—*Acutus* ♀ × *miliaris* ♂. First division, showing narrow spindles, and in fig. 22, two vesicles. Not all the chromosomes shown.

Figs. 24, 25.—*Acutus* ♀ × *miliaris* ♂. Two spindles showing small vesicles. Fig. 25 combined from two sections.

Fig. 26, *a, b, c*.—*Acutus* ♀ × *miliaris* ♂. Chromosomes in three successive sections of a monaster; 36 chromosomes.

Fig. 27, *a, b*.—*Acutus* ♀ × *miliaris* ♂. Two anaphase groups in face, from one spindle; 37 chromosomes in 27 *a*, 31 in 27 *b*.

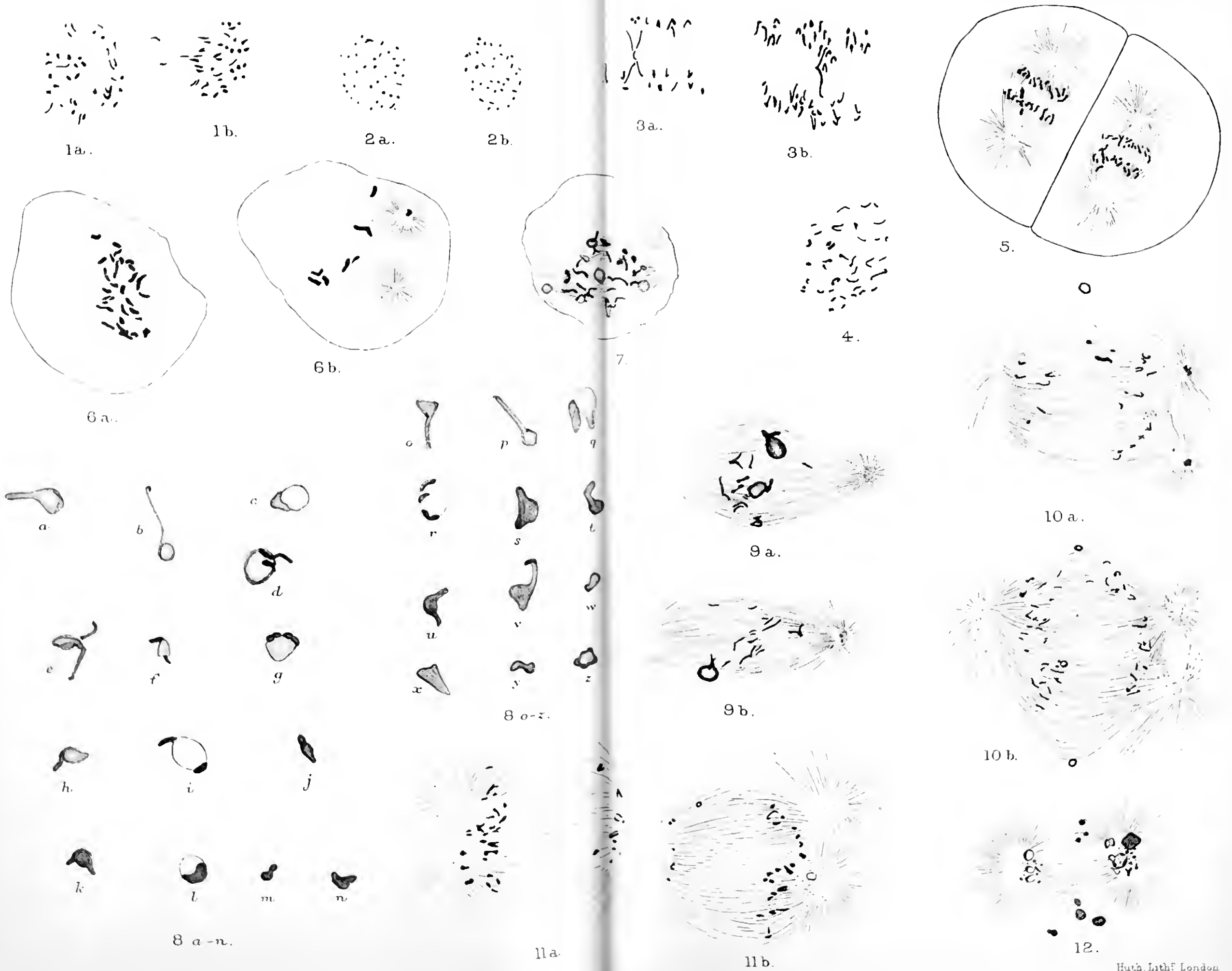
Fig. 28.—*Esculentus* ♀ × *miliaris* ♂. Anaphase group in face; 36 chromosomes.

Fig. 29.—*Miliaris* ♀ × *esculentus* ♂. Telophase, first division. Irregular groups at poles, three or four chromosomes eliminated. Combined from four sections.

Fig. 30.—*Miliaris* ♀ × *acutus* ♂. Two-cell stage. (Each cell was in metaphase of second division; section through the poles.) Three chromosomes eliminated (one drawn from the next section).





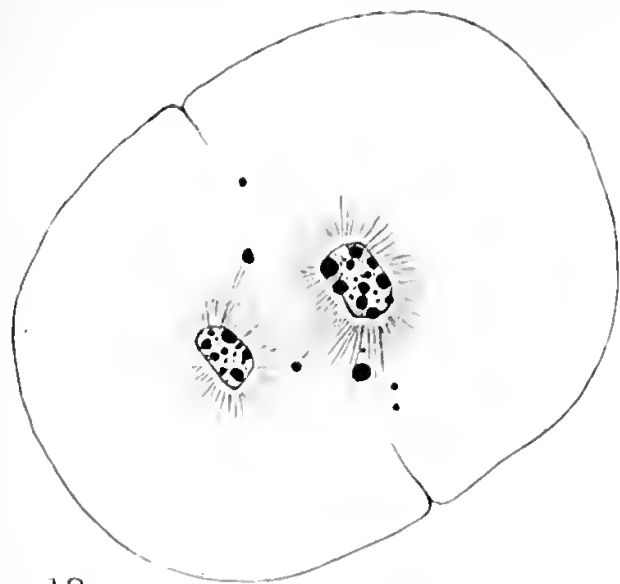




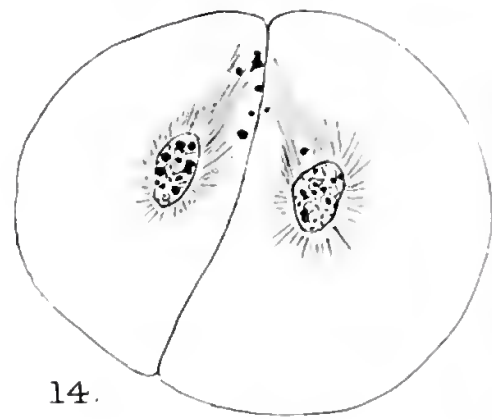








13.



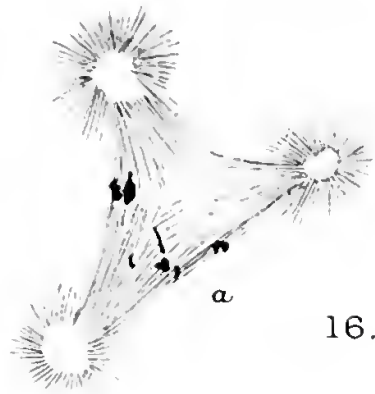
14.



a



b



a



b



a



b



c

17.

16.

15.

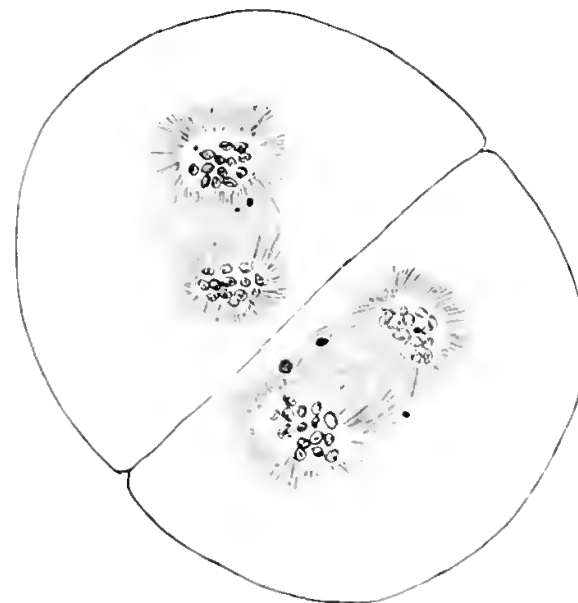


a

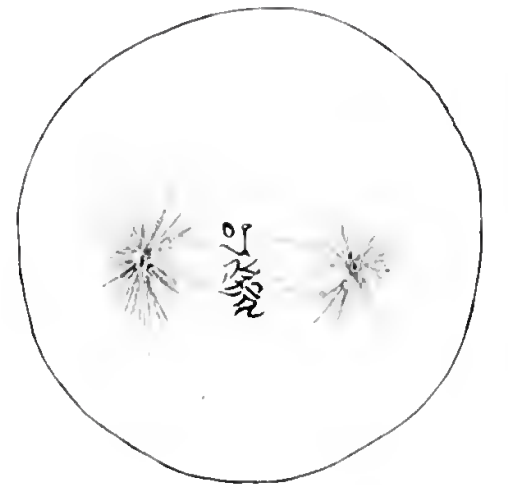


b

21.



20.



22.



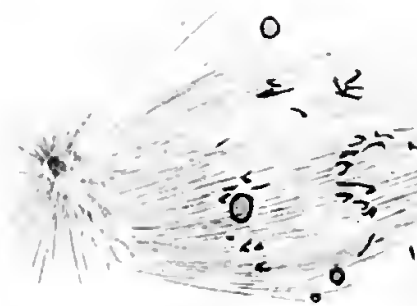
18 a.



18 b.



19 a.



19 b.

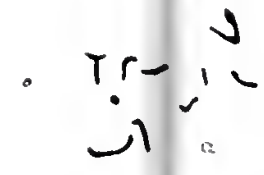


a



b

27.



a

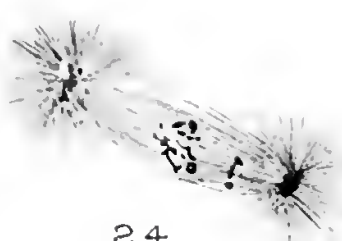


b



c

26



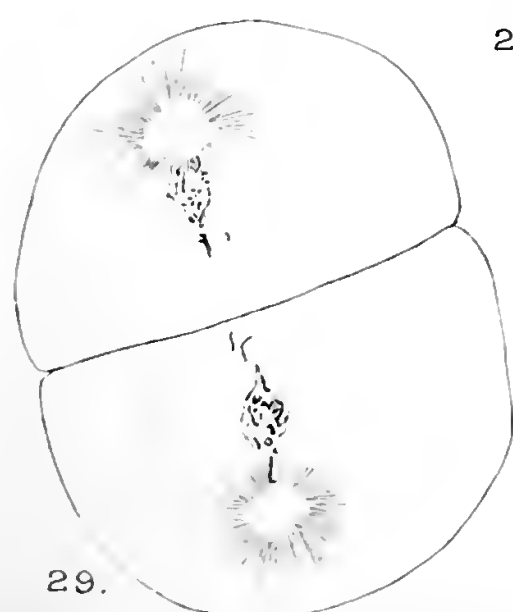
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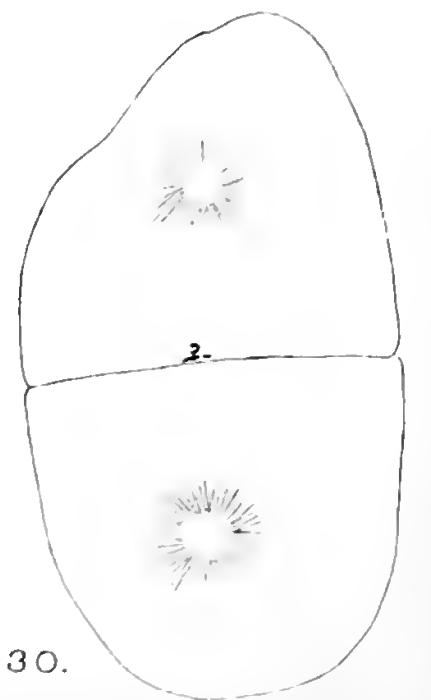
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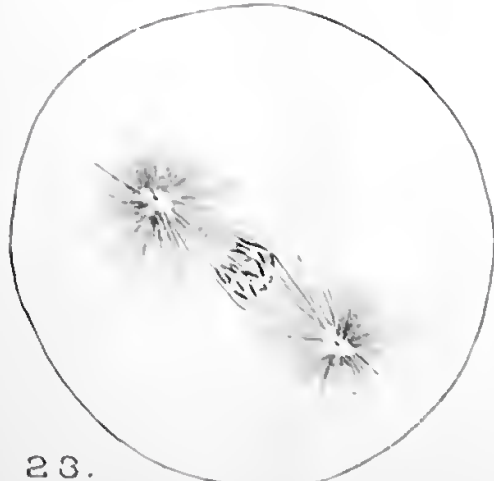
28.



29.



30.



23.



## The Life-Cycle of *Moina rectirostris*.

By

**The late G. H. Grosvenor, M.A.,**

New College, Oxford,

and

**Geoffrey Smith, M.A.,**

New College, Oxford.

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IN the summer of 1904 the late Mr. G. H. Grosvenor began some experimental observations on the life-cycle of *Moina*, with the object of testing Weismann's hypothesis (1) that the succession of parthenogenetic and sexual individuals is controlled by an internal rhythm independently of external circumstances. The species of the genus *Moina*, as pointed out by Weismann, belong to the polycyclic group of Cladocera which inhabit small ponds and which produce sexual individuals with great frequency, and this fact, together with the ease with which they can be cultivated, renders them convenient for experimental purposes.

After cultivating the animals for some time, Grosvenor noticed that if the parthenogenetic females were kept isolated in separate vessels from the time of their birth to the period at which they produced young, they gave rise to a much smaller proportion of males than was the case when they were kept crowded together in the same vessel. His method of cultivation was to place the young newly hatched parthenogenetic females, either isolated or together in numbers of about five to fifteen, in ordinary tumblers three quarters full of tap-water, and to add to the water a small quantity of a stock infusion

made from dry horse-dung. The actual numbers obtained by him in these early experiments were as follows:

*Moina rectirostris*: Oxford, 1904. June, July and October.

Isolated.		Crowded.	
No. of ♂s.	No. of ♀s.	No. of ♂s.	No. of ♀s.
4	95	50	153
4·2% per cent. males.		24·6 per cent. males.	

*Moina macrocopa*: Oxford, 1904. June and July.

Isolated.		Crowded.	
No. of ♂s.	No. of ♀s.	No. of ♂s.	No. of ♀s.
25	224	41	92
10% per cent. males.		30·8% per cent. males.	

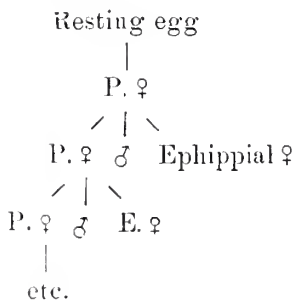
From these figures it will be seen that when the parthenogenetic females were kept isolated they produced 4·2 per cent. and 10 per cent. males, but that when they were kept crowded together but otherwise under identical conditions they produced 24·6 per cent. and 30·8 per cent. males.

In 1906 Grosvenor asked me to join him in repeating and extending this investigation, and during the summer and autumn of that year we bred *Moina rectirostris* on a large scale and subjected them not only to the conditions of isolation and crowding, but also to different temperatures. We obtained the *Moina* from a small pond at Sutton near Stalham on the Norfolk Broads, and most of the experiments were done in the fresh-water laboratory belonging to Sir Eustace and Mr. Robert Gurney, to whom we offer our thanks for giving us every accommodation in their laboratory.

The result of these experiments was to show that Grosvenor's idea as to the factor of isolation and crowding influencing the life-cycle was well founded, and also to prove that the influence of temperature, especially when combined with isolation, was of great importance. We were, in fact, able by keeping the parthenogenetic females isolated in an incubator at about 28° C. to inhibit absolutely and for an indefinite period the production of sexual forms. Although this latter result was of a far more definite kind than could be drawn from any experiments published at the time or since, we refrained from publishing them, as we hoped to find out what the exact nature of the factor of isolation and crowding might be, but the experiments which we made to settle this point were inconclusive. Owing to other duties and preoccupations the work lapsed, but Mr. Grosvenor's sudden death makes it desirable to publish the results as they stand.

Before referring to, and explaining, the tabular statement of our results, it is necessary to make some general observations on the life-cycle of *Moina rectirostris*.

The fertilised egg, after lying dormant, invariably hatches out as a female, which reproduces parthenogenetically. The parthenogenetically produced young may be either themselves parthenogenetic females, or they may be males or ephippial females. The parthenogenetic females of this generation, again, may produce all three kinds of individuals, and so on indefinitely. Schematically we may represent this type of life-history as follows :





In fact, as Weismann pointed out, there is only one generation which invariably and uniformly consists of parthenogenetic females, viz. the first generation which hatches out from the resting eggs. Any of the succeeding generations produced parthenogenetically may consist of males and ephippial females as well as of parthenogenetic females, but that they invariably are so constituted is negated by our experiments.

The ephippial or sexual females and their relation to the parthenogenetic females demand a word of explanation. The two kinds of female do not differ from one another structurally at first, but a young female that is destined to produce a resting egg and ephippium can be soon recognised by the greater opacity of the ovary, and later by the presence of the large resting egg in the ovary. At most two such resting eggs are brought to maturity and deposited in the specially prepared brood-pouch or ephippium, where they may be seen as opaque dark-red bodies of very large size. The ordinary eggs of the parthenogenetic female are, on the other hand, very numerous, small, and of a transparent greenish colour. Now, as the result of our observations, it is found that an ephippial female, if it is not fertilised by a male, may reabsorb its resting eggs and turn into a parthenogenetic female later. But we did not find any case of a female that had produced eggs parthenogenetically turning into an ephippial female, and giving rise to resting eggs. This is in agreement with Weismann's observations on Cladocera in general, Issakowitsch (2) being the only observer who records the contrary in the case of *Simocephalus vetulus*.

In the tables given on p. 521, 522, no mention is made of the occurrence of ephippial females, because it was impracticable to include them by the methods necessary for dealing with such large numbers of individuals, owing to the necessity of keeping the females alive for some time in order to determine whether they were ephippial or not. Nevertheless this was done in a large number of cases, and it was found that ephippial females occurred in the same broods in

which males were being produced, so that it appears that the conditions which call forth the production of males are the same as those which determine the production of ephippial as opposed to parthenogenetic females.

A good deal of confusion has arisen in regard to the life-cycle of Cladocera by the tacit assumption that the conditions controlling the production of males are sex-determining conditions—that alterations in the food supply, temperature, etc., condition the production of males as opposed to females.

This is an altogether mistaken view. Alterations in the external conditions do not alter the sex-ratio, except in so far as they may change the method of reproduction from that by means of parthenogenetic females to that by means of sexual males and females. For instance, in our own experiments isolation in the warmth led to the entire suppression, not only of males, but also of sexual females, while crowding at room temperature or in the cold led to the increase of males and ephippial females as opposed to parthenogenetic females. Thus, what is really shown to be influenced by the external conditions is not the sex-ratio, but the production or suppression of the sexual forms of both sexes.

The same stricture applies to all the experimental work on the life-history of the Cladocera, which is sometimes loosely termed work on sex-determination, but which is really calculated to throw light, not on the determination of sex, but on the alternation of asexual with the sexual mode of reproduction. Since in our experiments the same conditions called forth the production of males and sexual females, we can only conclude that these conditions were not specific sex-determining conditions, but simply determined that sexual males and females should be produced as opposed to parthenogenetic females. It is, of course, possible that external conditions, besides influencing the occurrence of sexual as opposed to asexual reproduction, may also influence the proportions of males to sexual females in any brood, but our experiments do not throw any light on this point, and the experiments of other observers appear to be beset by the

same limitation. The main results of our experiments are given in tabular form on p. 521, 522.

Table I gives the actual numbers of the males and females produced by parthenogenetic females when isolated in separate vessels and when crowded together in the incubator between  $25^{\circ}$  and  $30^{\circ}$  C. (column H) ; at room temperature, about  $14^{\circ}$  C. (R) ; and in an ice-chest between roughly  $8^{\circ}$  and  $1^{\circ}$  C. (C). In all, ten experiments are included, Nos. 2 to 12 in the first vertical column. These numbers refer to the particular females which were used as the original parents to start the lines of descent which were employed in the experiments. In the course of the experiments the actual pedigrees of every female used was known, but in Table I all the offspring of succeeding generations are summed and entered together according to whether they were produced from an isolated or "crowded" parent at room temperature, in the incubator or ice-chest. The offspring of the ten different lines are, however, kept separate in the horizontal lines. The first total given in the first horizontal line below the table gives the grand total of males and females produced by all the different lines.

Thus the isolated females in the incubator gave rise to 1167 individuals, all of which were females, no males being produced. The isolated females at room temperature gave 323 males to 1385 females, or 19.1 per cent. males, those in the cold chamber 10 males to 174 females, or 5.4 per cent. males.

The crowded females in the incubator gave 286 males to 657 females, or 30.3 per cent. males ; those at room temperature gave 1631 males to 1487 females, or 52.3 per cent. males ; and those in the cold chamber gave 167 males to 226 or 42.5 per cent. males.

The most striking of these results is the entire absence of males in the broods of females kept isolated in the incubator. This occurred in six different lines of descent in which parallel cultures at other temperatures, or when the parents were crowded, gave a large proportion of males, so that it cannot be argued that special female-producing lines were by

chance chosen for these experiments.<sup>1</sup> The suppression of the sexual forms must therefore be certainly attributed to the condition of high temperature and isolation of the parents.

In line 5 eight successive generations, containing 413 individuals, were propagated without the appearance of sexual forms.

The effect of isolation is also seen in the fact that the isolated parents at room temperature and in the cold chamber gave a smaller proportion of males than when the parents were crowded under the same conditions, viz. 19.1 per cent. and 5.4 per cent. males as against 52.3 per cent. and 42.5 per cent.

The effect of temperature by itself is seen in the fact that when the parents were isolated in the incubator they gave 0 per cent. males, while isolation at room temperature gave 19.1 per cent. males; also crowding in the incubator gave 30.3 per cent. males, while crowding at room temperature gave 52.3 per cent.

The effect of low temperature, i. e. in the cold chamber at about 5° C., gave actually a lower percentage of males than at room temperature both when the parents were isolated and when they were crowded. The numbers of broods and of individuals produced under these conditions were small, as it was found that the extremely low temperature inhibited growth and reproduction. It might be expected *à priori* that since more males are produced at room temperature than in the incubator, a still greater proportion of males would be produced in the cold, but it appears that the factor of extreme cold introduces another element—possibly that of very slow growth—into the conditions, which acts in an opposite direction.

The influence of isolation and of crowding can be shown by another method of arranging the results. In Table II are given

<sup>1</sup> Prof. R. C. Punnett, in his work on "Hydatina," has suggested that such female-producing lines exist. Owing to the way in which our experiments were performed, viz. by subjecting the individual members of each brood to different conditions, it is impossible to account for our results in this way. We are, however, not in a position to deny that it may be possible to segregate out a pure female-producing line in *Moina* in accordance with Punnett's suggestion.

the proportions of males and females produced at room temperature according to the number of parents present in the vessel. The number of parents kept in the same vessel varied from one to thirty-four. It cannot be said that the proportion of males to females steadily rises as the degree of crowding of the parents increases, as the numbers fluctuate rather erratically, but by grouping the numbers together in various ways it can be shown that on the whole the proportion of males does increase with an increased intensity of crowding. For instance, to take the largest grouping, when one parent was present 19.1 per cent. males were produced, when two to seven parents 37.3 per cent. males, when eight to thirteen parents 60.9 per cent. males, and when fourteen to thirty-four 65.2 per cent. males. We may take it, therefore, as established that the two factors of crowding and temperature profoundly influence the production of the sexual forms, and that by isolation of the parents at a temperature of about 28°C. it is possible to suppress entirely this production.

The inquiry as to how these factors of crowding and temperature brought about this effect was found to be beset with great difficulties. It seemed probable at first that the effect of crowding was due to the accumulation of excretory matter in the glasses, but experiments in which isolated females were placed in culture-water which had previously contained numerous individuals of *Moina* gave negative results, the isolated females in the incubator in such culture-water producing invariably females. Culture-water which had contained great numbers of *Moina* was also evaporated down to dryness and the residue dissolved in fresh culture-water, but again with negative results. It is, of course, possible that the excretory matter is of an unstable character, and quickly oxidised or destroyed as soon as it is formed. There is, however, another possibility, viz. that the effect of crowding is to lessen the food supply for each individual. The food of *Moina* consists of the organisms in the infusion, but it is uncertain whether the Cladoceran can feed indifferently on all the bacteria and infusoria found in such infusions, or

whether they are confined to some particular kind of food. If they are omnivorous it is inconceivable that the presence of eight or nine individuals in our culture-glasses should cause any shortage of food, but if they really confine themselves to some particular organism in the infusion as food it is very probable that there is a limited supply of this organism, and that the presence of several *Moina* is sufficient to upset the balance. From the behaviour of *Moina* in the culture-medium it would seem that they are actively hunting some special prey, and the peculiarly local occurrence of the various kinds of *Cladocera* points to their being in general dependent on special organisms for food. There can be no doubt that an important step forward will be taken when we can settle this question and cultivate these *Cladocera* on relatively pure cultures of their appropriate food. When the organisms are cultivated on the mixed and complicated fauna of an infusion, as was the case in our experiments, it is impossible to regulate the food supply, as there is no means of controlling the relative abundance of the particular constituent which alone may be serving as food.

For the present we must be content to say that under certain optimum conditions, which can be attained by isolation and a moderately high temperature, it is possible to propagate *Moina* entirely by parthenogenesis without any production of sexual forms, but whether this result is due to the absence of excretory matters or to an abundant food supply it is impossible to decide.

Although our results are incomplete in this direction, yet we are able to furnish a definitely negative answer as to the truth of Weismann's contention that the life-cycle is an hereditarily fixed process which runs its course independently of external conditions. It is, however, by no means certain that the life-cycles of other *Cladocera* are as sensitive to external conditions as *Moina*. It may be found possible to inhibit the production of sexual forms indefinitely in other species of *Cladocera*, but it seems probable that the artificial production of sexual forms in any particular generation of those forms

which normally produce only one or two epidemics of sexual forms in the year may be beyond the power of the experimenter, and may be dependent on an internal rhythm such as Weisman suggests. Probably for each species of Cladocera this internal rhythm exists, by which the parthenogenetic or sexual nature of the successive generations is determined, but it is now certain that this "determination" is of a plastic nature, and subject to radical modification in response to changing conditions. The degree of plasticity, and the extent to which the normal life-cycle may be modified, probably differs very greatly in different species. Scharfenberg (3), who has recently worked with *Daphnia magna*, admits the influence of external conditions, but holds that the life-cycle is fairly rigidly determined in Weismann's sense. Issakowitsch, on the contrary, holds that nutritive conditions have a preponderating influence on the life-cycle, and the earlier experiments of Kerhevé point in the same direction.

A critical discussion of the literature (see Scharfenberg [3]), is, however, better postponed until further experiments have been performed, and especially the nature of the food which the various kinds of Cladocera are dependent on has been thoroughly investigated.

#### SUMMARY.

(1) According to Weismann, in the life-cycle of *Moina* sexual forms should be produced in every parthenogenetic generation independently of external conditions.

(2) By isolating the parthenogenetic females at birth until the production of the brood at a temperature of 25° to 30° C. the production of sexual forms is entirely suppressed, 1167 parthenogenetic females and no sexual forms having been obtained by this means.

(3) Parallel cultures with related females crowded together in the culture-glasses at a temperature of 25° to 30° C. gave 30·3 per cent. males, and at about 14° C. gave 52·3 per cent. males. Isolated females at 14° C. gave 19·1 per cent. males.



Isolated females in an ice chest at about 5°C. gave 5.4 per cent. males, when crowded together 42.5 per cent.

(4) The intensity of crowding, measured by the number of parents kept together in the same glass, is shown to have a not very constant effect on the proportions of males produced, on the whole the proportion of males increasing with the intensity of crowding.

(5) The influence of isolation and of a high temperature on the suppression of the sexual forms may be ascribed either to the comparative absence of excretory matter under these conditions, or else to the nutritive conditions being under these circumstances highly favourable.

TABLE I.—Number of Males and Females in Broods produced at Different Temperatures in Individual lines.

Line.	Isolated.						Crowded.					
	H.		R.		C.		H.		R.		C.	
	♂ s.	♀ s.	♂ s.	♀ s.	♂ s.	♀ s.	♂ s.	♀ s.	♂ s.	♀ s.	♂ s.	♀ s.
2.	—	215	58	174	—	—	63	291	282	290	—	28
	0°/o	—	25°/o	—	—	—	17.7°/o	—	49.3°/o	—	0°/o	—
3.	—	—	28	25	—	—	—	—	12	22	—	—
	—	—	52.8°/o	—	—	—	—	—	35.2°/o	—	—	—
4.	—	—	8	57	—	—	—	—	122	127	13	15
	—	—	12.3°/o	—	—	—	—	—	48.9°/o	—	46.4°/o	—
5.	—	419	—	24	1	153	2	73	36	35	—	11
	0°/o	—	0°/o	—	5°/o	—	2.6°/o	—	50.7°/o	—	0°/o	—
6.	—	—	78	567	4	4	—	—	592	405	41	50
	—	—	12.1°/o	—	50°/o	—	—	—	59.3°/o	—	45°/o	—
7.	—	98	58	145	—	—	—	—	151	191	28	16
	0°/o	—	28.5°/o	—	—	—	—	—	44.1°/o	—	63.3°/o	—
9.	—	62	8	54	—	—	86	—	63	160	—	—
	0°/o	—	12.9°/o	—	—	—	100°/o	—	28.2°/o	—	—	—
10.	—	87	65	130	—	—	—	—	313	134	—	—
	0°/o	—	33.3°/o	—	—	—	—	—	70°/o	—	—	—
11.	—	286	2	159	—	—	41	199	54	123	76	79
	0°/o	—	1.2°/o	—	—	—	17.1°/o	—	30.5°/o	—	49°/o	—
12.	—	—	18	50	5	17	94	94	6	—	9	27
	—	—	26.5°/o	—	21.7°/o	—	50°/o	—	100°/o	—	36°/o	—
Total	0	1167	323	1385	10	174	286	657	1631	1487	167	226
	0°/o	—	19.1°/o	—	5.4°/o	—	30.3°/o	—	52.3°/o	—	42.5°/o	—



**On Methods of Producing Artificial Parthenogenesis in *Echinus esculentus* and the Rearing of the Parthenogenetic plutei through Metamorphosis.**

By

**Cresswell Shearer,**

University Lecturer in Experimental Morphology, Cambridge,

and

**Dorothy Jordan Lloyd,**

Bathurst Student, Newnham College, Cambridge.

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With Plates 30 to 32.

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I. INTRODUCTION.

DURING the last seven or eight years extensive experiments have been carried on in the laboratory of the Marine Biological Association in rearing various marine larvæ. In particular, attention has been directed to perfecting means for bringing the delicate plutei of Echinoderms through metamorphosis. As the result of considerable investigation a method has been found by which these larvæ can be reared with considerable ease and certainty. We have already successfully raised at Plymouth several species of sea-urchins and their hybrid crosses to the adult condition.

This suggested to us that these methods might equally be

applied to rearing the parthenogenetic plutei obtained by some of the various ways of bringing about the development of the Echinoderm egg, such as that of Loeb, Delage and other investigators. The following paper is a record of some experiments that have been carried on during the past two seasons at Plymouth, in an endeavour to accomplish this object.

The first aim of our work was that of devising a convenient method for obtaining large numbers of parthenogenetic plutei; the first year, therefore, we confined our attention to a thorough trial of the well-established method of Loeb.

Little success has attended the attempts of those who have sought to repeat Loeb's (17) work on inducing artificial parthenogenesis of the eggs of the Echinoderms of our coast. On this account Prof. Loeb, while in England (1909), visited Plymouth, and made a number of experiments. He has handed over his data to us, with a request to determine the optimum times and strengths of solutions suitable to the peculiar conditions presented by the low alkalinity of the sea-water of the British coast.

In working at Plymouth, it is first necessary to raise the alkalinity of the sea-water prior to the treatment of the eggs by hypertonic solution.

In the second year we tried several methods, and finally adopted a combination of Loeb's and Delage's tannin and ammonia method.

Our experiments have shown that parthenogenetic plutei obtained by Loeb's improved method can be easily reared to a late stage and through metamorphosis. We have been unsuccessful, however, in getting the young Echini to live any length of time after metamorphosis, and few of our parthenogenetic Echini have grown to any considerable size.

The very high percentage of larvæ which we have raised to a late stage (twenty-five to thirty days) renders our results of interest. In some of our culture-jars we have had as many plutei go through to a late stage and form Echinusrudiments, as would do so in a lot of eggs normally fertilised

with sperm. This clearly shows that the growth of the parthenogenetic plutei to the sexually mature stage is well within the range of possibility if sufficient time and care be taken with them.

Delage (5) has been the only investigator who has attempted to raise parthenogenetic plutei to a late stage. He obtained in all some six completely metamorphosed urchins of *Strongylocentrotus lividus*. Two of these attained considerable size, and one finally developed rudimentary gonad cells. Three of these six were obtained by his HCl and ammonia method, and three were derived from his tannin and ammonia method.

With the exception of this work of Delage (5), no attempt has been made to rear parthenogenetic plutei, and their characters have not been closely compared with those of the normal larvæ. In our work we have carefully compared the growth of the two at different ages, from the blastula to the metamorphosed Echinus stage. This comparison has clearly established the fact, that there is always a slight difference; and that once the features that characterise both are known, it is never possible to mistake one for the other. That there should be a slight difference between the two is not unlooked for, when we consider that it has now been established that the parthenogenetic larvæ, in the early stages at least, develop only in the presence of the reduced number of chromosomes. (Hindle [6] and Wilson [21].)

These differences are most marked in the length and shape of the arms and in the pigmentation. In the later stages (eight-armed pluteus) the parthenogenetic are always distinguishable from the normal larvæ, by a slight granular condition of the protoplasm. The relative rates of growth differ greatly, development of the parthenogenetic larvæ being much more rapid in the early stages up to the four- and eight-armed pluteus, while after this it is much slower than in the normally fertilised ones. While the normal larvæ usually metamorphose in five to six weeks from the time of fertilisation, none of our parthenogenetic larvæ have meta-

morphosed within a shorter time than eight weeks, and usually have taken at least ten weeks. Again, the development of the parthenogenetic larvæ is never as regular as that of the normal; the arms tend to grow somewhat irregularly from the first, as a glance at the figures will show. It is remarkable, however, that a large number of these irregular larvæ are quite healthy, and form perfect *Echinus* rudiments, and may metamorphose into young sea-urchins. While in the normal larvæ, abnormality of growth is usually a sign of early death and inability of the larvæ to reach a late stage, in the parthenogenetic, it seems to have no such significance. The irregularity of these larvæ seems most marked in the order in which the arms appear. This often gives them a peculiarly abnormal appearance. It has to be borne in mind, however, that this is sometimes seen in healthy larvæ obtained from tow-nettings, and is, perhaps, of no great significance. Another feature presented by the parthenogenetic plutei, is that once they have begun to form their *Echinus*-rudiments, the rest of the pluteus rapidly degenerates and begins to lose its arms. Thus, by the end of the third or fourth week, the parthenogenetic plutei are quite different in appearance from the normal ones, in that their arms have already undergone considerable degeneration, while their *Echinus*-rudiments are still small; the normal larvæ of the same age have perfect arms, and large *Echinus*-rudiments. A frequent abnormality of the parthenogenetic larvæ is a peculiar crumpling in of the dome of the pluteus, shown in fig. 22. This abnormality has appeared very often in our cultures.

The remarkable rate of growth of the parthenogenetic plutei in the early stages is in marked contrast to their slow growth in the later stages. As the time for metamorphosis approaches they become more and more feeble, and in the last stage of metamorphosis they seem unable to absorb the remnants of the pluteus. They may remain in this condition for weeks, until they finally die from the interference of the still attached remains of the pluteus. The spines and pedicellariæ can be seen protruding through the

pluteus skin, but in nearly all cases the young *Echinus* fails in the end to absorb this completely, and remains indefinitely in this condition till it dies.

As the mouth and anus of the young sea-urchin do not appear till about the end of the first week after metamorphosis, the inability of the larvæ to get rid of the remnants of the pluteus so unduly lengthens the process of metamorphosis, that they seem unable to live the length of time required afterwards, until the mouth and anus form, and they are able to take food. This would seem to be the explanation of the almost invariable death of the larvæ during metamorphosis. As we have said, large numbers of our parthenogenetic plutei reached this stage, and then apparently died of starvation. We have been unable to get them to metamorphose more quickly, although several changes of food were tried in the earlier stages, in the hope that this might give better results. If the larval rate of growth could be accelerated slightly at this point, in metamorphosis, parthenogenetic young *Echini* could be obtained probably as readily as the normally fertilised ones.

In the use of sea-water in all our experiments we have observed certain precautions to prevent any possible infection by sperm. From experiment we have found that in no case do the sperm of *Echinus* at Plymouth survive or remain alive after standing for more than thirty-six hours. We have used water collected in large sterilised carboys (3-4 gallons capacity) from some seven miles out from the Plymouth break-water and then brought into the laboratory and allowed to stand for a week or more before being used. We have never had any of our controls go wrong from the use of this water, and therefore we have concluded that any possible infection from it has been avoided. Water collected in this way and allowed to stand in the above-described manner, we call "outside water." This outside water never seems to contain any plutei as apparently did the water used by Delage (5), so we have not resorted to filtering it to prevent any contamination from this source.



The true alkalinity of the "outside water," that is, the concentration of the hydrogen ion, expressed in gram-equivalents per litre, has been determined by means of the colorimetric method of Sørensen<sup>1</sup> by Mr. D. J. Matthews. He has kindly given us the following figures for the alkalinity during the two years covered by our work. In the latter part of 1911, the "outside water" gave a distinct red colour with phenolphthalein, and  $P_H$  was consequently above 8.0, generally 8.15 to 8.25. In the spring of 1912 it gave no colour with phenolphthalein, but tests with  $\alpha$ -naphtholphthalein showed that  $P_H$  was about 7.9, but this figure gradually increased as the season advanced until it was about the same as in the previous autumn.

By Berkefeld water we mean ordinary laboratory tank-water, which has been treated with animal charcoal, aerated and filtered through a Berkefeld filter and then stored in sterilised flasks. This water is free from bacteria and infusoria, which increase in such great numbers in ordinary "outside water" when this has stood some time in the laboratory; and it is of considerably lower alkalinity than "outside water." In Berkefeld water we have noticed that the segmentation of the egg is somewhat slower in the early stages than it is in "outside water," and possibly this is due to the low alkalinity of the "Berkefeld" as compared with the "outside water," the average alkalinity of the "Berkefeld" water being about  $P_H = 7.40$  as compared with  $P_H = 8.15$  for "outside water." The amount of ammonia in both is roughly the same. We

<sup>1</sup> According to Sørensen's determination the dissociation constant of pure water is  $10^{-14.14}$  at 18° C., and a litre of pure water at this temperature would contain  $10^{-7.07}$  gram-equivalents of hydrogen ions, and the same number of hydroxyl ions. He expresses, for convenience, the acidity of a solution by the symbol  $P_H$ , which is the numerical value of the exponent of the concentration of the hydrogen ion with the sign changed. Thus for pure neutral water  $P_H = 7.07$ . Owing to the change of sign, the higher the value of  $P_H$  the lower the acidity or the greater the alkalinity. (See Palitzsch, "Über die Messung der Wasserstoffionkonzentration des Meerwassers," 'Publ. de Circonstance No. 60. Conseil Permanent Internat. pour l'exploration de la Mer,' Copenhagen, 1911).

have used almost exclusively "outside water" for the early stages of all our experiments.

When the eggs, after treatment with hypertonic sea-water, have been returned to normal sea-water and have segmented and developed to the actively swimming blastula stage, they have been pipetted off, and placed in breffets<sup>1</sup> of Berkefeld water. In this way any contamination of our cultures from the outside water has been avoided as far as possible, any contamination also being watched by proper controls made with every experiment.

The stock solutions of our reagents used have been made from doubly re-crystallised salts dissolved in water re-distilled from glass.

The hypertonic sodium-chloride solution was tested by precipitation with silver nitrate.

The solutions used in the experiments were made by adding quantities of the stock solutions to sterile sea-water as they were required.

In all our operations proper attention has been paid to sterilisation of instruments, pipettes, glassware, etc. Each sea-urchin, before being opened, was placed under a tap of fresh water to kill any sperm on its surface, and after each one opened the hands and instruments have been re-sterilised.

Of every experiment made a proper control has been kept, as well as every batch of eggs being tested by normal fertilisation. In any case where the control has gone wrong, or the eggs failed to fertilise in the normal way when sperm was added to the fertilisation control, the entire batch of eggs was thrown away, and the experiment discontinued.

The method we have followed in rearing our larvæ is that elaborated by Allen and Nelson (1), and which has been already extensively applied by one of us (20) to the rearing of Echinoid hybrids. It is therefore unnecessary to describe this method here, as a detailed description has already been published by Allen and Nelson (1). We have made use of pure cultures of diatoms to feed the parthenogenetic plutei,

<sup>1</sup> Glass jars of 2500 c.c. capacity.

and as far as this is concerned, have not found that they need any special food other than that used for feeding the normal fertilised larvæ. Our young urchins have in most cases, as we have said, failed to develop their mouths sufficiently soon after metamorphosis to feed on the algæ we have found so satisfactory in our Echinoderm hybridisation experiments.

One remarkable fact which we have repeatedly noticed is that the eggs that develop and segment best, after treatment with various chemical solutions, are not those that are in the very best condition for fertilisation with sperm. In our experiments in Echinoderm hybridisation we have learnt to distinguish what are more or less the ripest eggs for giving the best results on fertilisation with sperm. This consists in the absence of nucleus and a peculiar appearance of the cytoplasm, which, although somewhat hard to describe, is readily learnt from practical observation. We have been surprised, therefore, that invariably those eggs, which we concluded from our observations were in the best condition for giving the highest percentage of normal fertilisation with sperm, were just those that gave poor results by methods of artificial parthenogenesis. We have repeatedly remarked that those lots of eggs in which our fertilisation controls with sperm gave very high percentages, gave very poor results by any method of artificial parthenogenesis we might use. In fact, early in our work we soon learnt to distinguish the peculiar stage of ripeness necessary to give good results by artificial fertilisation, and this stage of ripeness is quite different from that which experience has taught us is most suitable for normal fertilisation with sperm. This has been so constant a feature in all our experiments that there is no doubt that it is a definite factor which should be taken into account in considering the nature of the chemical changes which accompany artificial parthenogenesis, at least under the conditions presented at Plymouth.

The breeding period of *E. esculentus* is confined at Plymouth to a relatively short period, extending from the end of February, in favourable years, to the first part of May.

While it is frequently possible to get good ripe females well into June, most of the females have shed their eggs by the middle of May.

The exact time when the breeding period may be said to reach its maximum, seems to vary very considerably, some years being much nearer the former than the latter part of the season. This variation is influenced by temperature and other conditions. Of the two years through which this work has been extended the first may be said to have been normal in that the breeding season reached its height at the usual time, in the middle of April. The fertilisation controls went well, and the growth of the larvæ was rapid and vigorous.

In the season of 1912, however, good ripe females were much harder to obtain, and often, although the eggs of some of these looked quite ripe, they failed to segment when fertilised with sperm. Moreover, in our hybridisation experiments of 1912 we found the same trouble. We have some evidence, then, for supposing that the second season was not so favourable as the first for our experiments.

In 1911 we had no difficulty in getting a large number of plutei to reach a late period, and quite a number to pass through metamorphosis to the young urchin stage. In the season of 1912 very few reached metamorphosis, and none of them advanced as far as in the previous year. This difference in the two years should be kept in mind in comparing the results of the two years.

It is possible that the combined method of Loeb and Delage that we adopted during the second season has not so far, for these reasons, had a fair trial. The fact that we did not obtain young urchins by this method, may have its explanation in our possibly using unfavourable material for the majority of our experiments. It should also be remembered that we have been equally unsuccessful this year with Loeb's method. Whatever the differences between the two seasons may have been, it affected only the later stages of development, for little difference could be noticed between the numbers of blastulae obtained, during the early stages of the

experiments, in the two years. If possible, a higher percentage of blastulæ was obtained during 1912 than in the previous year. They seemed healthy and vigorous in every way, but did not survive to metamorphose as did those of the previous season.

## II. LOEB'S $MgCl_2$ METHOD AND DELAGE'S $HCl$ METHOD.

Some preliminary experiments were tried with a few of the early methods that have been devised by Loeb (11) and Delage (3 and 5) for producing artificial parthenogenesis, with a view to finding out what results they would give with eggs at Plymouth.

A large percentage of eggs were successfully induced to segment somewhat irregularly with Loeb's  $MgCl_2$  method. In all cases the segmentation was highly irregular even in the first stages, and became progressively more irregular as cleavage advanced. A small percentage of the eggs ultimately developed into unhealthy-looking prism larvæ, and finally into very abnormal plutei (figs. 3 and 5).

The most satisfactory results were obtained by placing the eggs in the following solution :

50 c.c. 20/8 N  $MgCl_2$  + 50 c.c. sea-water  
for two hours, and then transferring them back to normal sea-water. About 5 per cent. of the eggs formed plutei. In all these cases plutei were very unhealthy, and at the end of four days had become highly abnormal (figs. 3 and 5), although some lived for a fortnight or more, by the end of which time they had degenerated into shapeless masses of cytoplasm.

Some very irregular blastulæ were also obtained by Delage's (3)  $HCl$  method, but these never progressed beyond the blastula stage.

As both the above methods gave little promise of being much use for our work, they were not carried beyond the preliminary stage, and were soon abandoned.

## III. LOEB'S IMPROVED METHOD (figs. 5-9, and 12, 14, 15 and 16).

The method elaborated by Loeb (17) for bringing about artificial parthenogenesis in the eggs of sea-urchins consists in first treating the unfertilised eggs with sea-water to which small quantities of butyric or any other fatty acid has been added, and afterwards with hypertonic sea-water. Owing to the fact that the sea-water at Plymouth has a lower concentration of OH ions than that of the water of the American coast, it is necessary, in order to obtain successful results by this method at Plymouth, to transfer the eggs from the butyric acid solution to sea-water in which the OH ion concentration has been raised by the addition of small quantities of NaOH. After lying in this hyper-alkaline sea-water for a short time the eggs are changed to the hypertonic solution, and on being returned to normal sea-water segmentation takes place.

We have tried various strengths of butyric acid for longer and shorter times, as shown in the following table. The best membrane formation was brought about by using a mixture of 3 c.c. N/10 butyric acid and 50 c.c. of sea-water for 1.5 minutes.

TABLE I.

	Lot I.		Lot II	
	No membranes		5 <sup>2</sup> / <sub>10</sub> membranes.	
(1) 1 c.c. N/10 butyric acid + 50 c.c. sea-water for 1.5 min. . . . .	A few		—	
(2) Ditto, 2.0 min. . . . .	A few		—	
(3) Ditto, 2.5 min. . . . .	A few		—	
(4) 2 c.c. ditto, 1.5 min. . . . .	60 <sup>2</sup> / <sub>10</sub> poor		40 <sup>2</sup> / <sub>10</sub> ..	
(5) Ditto, 2.0 min. . . . .	80 <sup>2</sup> / <sub>10</sub> ..		—	
(6) Ditto, 2.5 min. . . . .	60 <sup>2</sup> / <sub>10</sub> ..		—	
(7) 3 c.c. ditto, 1.5 min. . . . .	70 <sup>2</sup> / <sub>10</sub> good		95 <sup>2</sup> / <sub>10</sub> good	
(8) Ditto, 2.0 min. . . . .	70 <sup>2</sup> / <sub>10</sub> poor		20 <sup>2</sup> / <sub>10</sub> ..	
(9) Ditto, 2.5 min. . . . .	70 <sup>2</sup> / <sub>10</sub> ..		25 <sup>2</sup> / <sub>10</sub> ..	
(10) Ditto, 3.0 min. . . . .	—		15 <sup>2</sup> / <sub>10</sub> ..	

Besides using membrane-formation as a criterion for the time and strength of the butyric acid, we also made experiments in which, after treating with butyric acid, the eggs are

passed through hyper-alkaline and hypertonic sea-water to normal sea-water. The degrees of segmentation at varying times in the butyric solution, other factors being kept constant, confirm the idea that times longer than about 1·5 minutes cause some damage to the eggs. Table II gives one series of our experimental numbers. The eggs after butyric acid treatment were washed thoroughly and transferred to—

- (1) 2 c.c. N/10 NaOH + 50 c.c. sea-water for 7 minutes.
- (2) 8 c.c. 2·5 M NaCl + 50 c.c. sea-water for 8 hours.
- (3) Normal sea-water.

TABLE II.

(1) 3 c.c. N/10 butyric acid + 50 c.c. sea-water for 1 min. . . . .	45% blastulæ.
(2) Ditto, 1·25 min. . . . .	50% "
(3) Ditto, 1·5 min. . . . .	35% "
(4) Ditto, 1·75 min. . . . .	60% "
(5) Ditto, 2 min. . . . .	10% "
(6) Ditto, 2·25 min. . . . .	5% "
(7) Ditto, 3·25 min. . . . .	No "

Note.—In (3) the low percentage of blastulæ may possibly be due to the eggs having only received one washing in sea-water after the butyric acid.

The alkaline sea-water into which the eggs were placed after membrane formation was tried in various strengths and for different times. The time factor did not seem to be of much importance within fairly wide limits (4–10 minutes), and as success in obtaining blastulæ is largely dependent on the rapidity of working, we ultimately adopted six minutes as a uniformly convenient time for exposing the eggs to its action. The strength of N/10 NaOH which gave us the best results for the seasons 1911 and 1912 was 2 c.c. in 50 c.c. of sea-water, but probably this factor will be found to vary from year to year. Table III gives some of our experimental numbers.

The eggs considered in Table III were treated with 3 c.c. N/10 butyric acid for 1·5 minutes and washed well before being placed in the NaOH solution. Afterwards they were



transferred for .75-1 hour to hypertonic sea-water (8 c.c. 2.5 M NaCl + 50 c.c. sea-water), and finally to normal sea-water.

TABLE III.

	Lot I, examined after a few hours.	Lot II, examined after 24 hours.	Lot III, examined after 24 hours.
(1) 0.0 c.c. N/10 NaOH + 50 c.c. sea-water for 6 min.	—	. 100% cytolyse	—
(2) .1 c.c. ditto . . .	. 40% dividing in irregular manner	—	—
(3) .2 c.c. ditto . . .	. 60% dividing	. 35% blastulæ	. 50% blastulæ.
(4) .3 c.c. ditto . . .	. 70% dividing	. 10% ..	. 5% ..
(5) .4 c.c. ditto . . .	. Division very irregular; many multi- polar asters	—	. 30% blastulæ.
(6) .5 c.c. ditto . . .	—	—	. 25% ..

Note.—Lot III (4) shows how one bowl of a series may for no apparent reason fall greatly below the standard of the others.

We have tried other alkalies, principally  $\text{Na}_2\text{CO}_3$ , as a means of raising the alkalinity of the sea-water, but we have found NaOH the most satisfactory. Table IV gives a comparison of the results obtained by the use of NaOH and  $\text{Na}_2\text{CO}_3$ .

TABLE IV.

	Lot I.	Lot II.
(1) .05 c.c. N/10 NaOH + 50 c.c. sea-water for 6 min.	. 50% blastulæ	—
(2) .1 c.c. ditto . . . . .	. 65% ..	—
(3) .2 c.c. ditto . . . . .	. 60% ..	. 30% blastulæ.
(4) .4 c.c. ditto . . . . .	. 50% ..	—
(5) 1.0 c.c. ditto . . . . .	—	. A few blastulæ.
(6) .05 c.c. N/10 $\text{Na}_2\text{CO}_3$ + 50 c.c. sea-water for 6 min. . . . .	. 50% blastulæ	—
(7) .1 c.c. ditto . . . . .	. 10% ..	—
(8) .2 c.c. ditto . . . . .	. 5% ..	. Segmentation very irregular.
(9) .4 c.c. ditto . . . . .	. None	—
(10) 1.0 c.c. ditto . . . . .	—	. No division.

After treatment with sea-water of raised alkalinity the eggs are put into hypertonic sea-water. For *Echinus esculentus* in general 8 c.c. 2.5 M NaCl + 50 c.c. sea-water for .75-1 hour gives the best results. These numbers are the same as those given by Loeb (17) as most satisfactory for the sea-urchins of the Californian coast. Both the strength of the hypertonic solution and the time during which the eggs are allowed to remain in it are of importance in bringing about parthenogenesis in many cases, but the experiments which we have made have convinced us that the condition of the eggs is the most important factor in determining whether parthenogenesis can, or cannot, be induced by chemical means. Frequently eggs are found to separate freely from the ovaries when the latter are shaken in water, and to appear ripe under the microscope, being spherical and having no visible nuclei. These may, however, fail to develop parthenogenetically, although varying strengths of hypertonic sea-water be used for different lengths of time. On the other hand, with some eggs (see Table VII below) 8 or 9 c.c. 2.5 M NaCl + 50 c.c. sea water appeared to act equally well.

In the results recorded in Table V the strength of the hypertonic solution used has been varied; in Table VI the time during which the eggs were exposed to its action; in Table VII both time and strength are variable. In all the experiments the eggs had previously been treated with butyric acid to cause membrane-formation and then with sea-water of raised hydroxyl ion concentration. In each series all conditions except those of the hypertonic sea-water were constant for the series.

TABLE V.

	Lot I.	Lot II.
(1) 4 c.c. 2.5 M NaCl + .	Very little attempt .	—
50 c.c. sea-water	at division	
for 45 min.		
(2) 5 c.c. ditto . . . .	—	. A few irregular attempts at cleavage.

TABLE V (*continued*).

	Lot I.	Lot II.
(3) 6 c.c. ditto . . . . .	Very irregular division, considerable cytolysis	A few irregular 64-cell stages.
(4) 7 c.c. ditto . . . . .	—	Large number of 64-cell stages, but irregular and flattened.
(5) 8 c.c. ditto . . . . .	Numerous swimming blastulae, 40 $\frac{2}{5}$	Large number of perfectly regular 64-cell stages, no cytolysis. 80 $\frac{2}{5}$ eggs attempted division and 50 $\frac{2}{5}$ formed blastulae.
(6) 9 c.c. ditto . . . . .	Considerable cytolysis, many egg-fragments. No blastulae	Good number of 64-cell stages, all rather irregular and tending to separate in groups.

TABLE VI.

	Lot I.	Lot II.
(1) 8 c.c. 2.5 M NaCl + 50 c.c. sea-water for 30 min.	Cleavage irregular. A few blastulae	10 $\frac{1}{5}$ normal blastulae.
(2) Ditto, 45 min.	50 $\frac{2}{5}$ blastulae	50 $\frac{2}{5}$ blastulae. Some half-blastulae.
(3) Ditto, 60 min.	A few blastulae	A few half-blastulae only.

TABLE VII.

(1) 7 c.c. 2.5 M NaCl + 50 c.c. sea-water for 30 min.	} Very few attempts at division.
(2) Ditto, 60 min.	
(3) 8 c.c. ditto, 30 min.	Few attempts at division.
(4) Ditto, 60 min.	80 $\frac{2}{5}$ swimming blastulae, 60 $\frac{2}{5}$ normal.
(5) 9 c.c. ditto, 30 min.	Few attempts at division.
(6) Ditto, 60 min.	80 $\frac{2}{5}$ swimming blastulae, 60 $\frac{2}{5}$ normal.

As the result of many experiments we have found that with Loeb's improved method the figures and procedure given below are generally the most satisfactory for *E. esculentus*.

(1) 3 c.c. N/10 butyric acid + 50 c.c. sea-water for 3 minutes.

(2) Wash in two or three changes of sea-water and transfer to :

(3) .2 c.c. N/10 NaOH + 50 c.c. sea-water for 6 minutes. Transfer to :

(4) 8 c.c. 2.5 M NaCl + 50 c.c. sea-water for .75-1 hour. Transfer to—

(5) Normal sea-water.

#### IV. DELAGE'S METHOD.

Delage (5) used tannic acid followed by ammonia to induce parthenogenesis in *Strongylocentrotus lividus* at Roscoff. Various media were used as bases for these reagents, but the one finally adopted was a mixture of 30 c.c. sea-water and 70 c.c. of a solution of cane-sugar containing 388 gm. per litre. Delage fixed on this strength of sugar solution, which is about 1.13 molecular, under the impression that it is isotonic with sea-water. Mr. D. J. Matthews, who has kindly determined the salinity of the "outside water" from one of the jars used in our experiments, gives us the figure  $S_{\infty} = 34.60$ . From Krummel's tables this gives an osmotic pressure of about 23 atmospheres, whereas the experimental number obtained by Lord Berkeley and Mr. Hartley (2) for the osmotic pressure of a solution of sucrose of the strength used by Delage is somewhere about forty atmospheres.

The tannic acid solution used by Delage contained 5.4 gm. of the acid per litre. In his paper he calls this  $\frac{N}{10}$  assuming that the carboxyl and five hydroxyl groups of tannic acid are all dissociated in solution. The dissociation constant for tannic acid has never been taken experimentally,

and since from the consideration of nearly allied organic acids it seems highly unlikely that tannic acid would even have the carboxyl group completely dissociated in the presence of such a weak base as ammonia, we have preferred to call the same strength of tannic acid M/60.

In his earlier experiments Delage added the eggs to a mixture of sea-water, sugar and tannic acid, leaving them for six minutes and then adding the ammonia, and after an hour transferring to normal sea-water. In his later experiments he added the ammonia before the eggs, merely placing these for an hour in a mixture of sugar, sea-water, tannic acid and ammonia. At Plymouth, with *E. esculentus* we have failed to get any results with this later method, and all our blastulæ have been raised by the earlier one. Table VIII gives a comparison of the results obtained by using the two methods on eggs from the same sea-urchin.

TABLE VIII.

## (1) Eggs were placed in—

(A) 15 c.c. sea-water + 35 c.c. 1·13 M sugar solution + 1·4 c.c. M/60 tannic acid, for 6 min.

(B) 1·5 c.c. N/10 ammonia were added to (A).

Eggs transferred to normal sea-water after

45 min. . . . . 5% blastulæ.

Ditto, after 60 min. . . . . 50% blastulæ.

Ditto, after 75 min. . . . . 30% blastulæ, but rather irregular.

## (2) Eggs were placed in:

15 c.c. sea-water + 35 c.c. 1·13 M sugar solution + 1·4 c.c. M/60 tannic acid + 1·5 c.c. N/10 ammonia.

Eggs transferred to normal sea-water

after 45 min. . . . . } Very few attempts

Ditto, after 60 min. . . . . } at cleavage. No

Ditto, after 75 min. . . . . } blastulæ.

The proportion of sea-water to 1·13 M cane-sugar solution, which is best for *E. esculentus* at Plymouth is 10 c.c. of the former to 40 of the latter. This is the strength which we have used in all our later experiments, though we have also

obtained good results with the proportion used by Delage, namely, 15 c.c. of sea-water to 35 c.c. of sugar. Table IX gives a series of experiments varying these proportions.

TABLE IX.

	Lot. I.	Lot II.
(1) 25 c.c. sea-water + 25 c.c. .	55% eggs showing attempt at cleavage. A few blastulæ.	50% eggs showing irregular cleavage.
1.13 M sugar solution,		
1.4 c.c. M/60 tannic acid		
for 6 min., 1.5 c.c. N/10		
ammonia for 60 min.		
(2) 15 c.c. sea-water + 35 c.c. .	15% blastulæ. .	Cleavage fairly regular. 25% blastulæ.
ditto	More cytolysis.	
(3) 10 c.c. sea-water + 40 c.c. .	50% good blastulæ	Cleavage less regular than in (2). 15% blastulæ.
ditto		
(4) 5 c.c. sea-water + 45 c.c. .	—	Very few attempts at cleavage.

The experiments given in Table X were made to vary the proportion of ammonia to tannic acid. It indicates that Delage's method of adding .1 c.c. more of ammonia than of tannic acid gives the best results. A higher proportion of ammonia is accompanied by increased cytolysis.

TABLE X.

(A) 15 c.c. sea-water + 35 c.c. 1.13 M sugar + 1.4 c.c. M/60 tannic acid for 6 mins.	
(B) (1) 1.4 c.c. N/10 ammonia added for 1 hr. .	70 % eggs showing cleavage, 30 % blastulæ.
(2) 1.5 c.c. ditto . . . . .	35 % blastulæ.
(3) 1.7 c.c. ditto . . . . .	Some superficial cytotoxicity, cleavage more irregular than (1) and (2). A few blastulæ.
(4) 1.9 c.c. ditto . . . . .	Large amount of cytotoxicity. Cleavage very irregular.

The proportion of tannic acid added to 50 c.c. of sea-water and sugar was also varied (see Table XI). In our later experiments we used 1.4 c.c. M/60 tannic acid followed by 1.5 c.c. N/10 ammonia to 50 c.c. of solution. The best time for allowing the reagents to act on the eggs is for one hour after adding the ammonia. Three quarters of an hour fails to stimulate many of the eggs to start cleavage, and an hour and a quarter materially increases the number of deformed blastulæ.

TABLE XI.—10 c.c. Sea-water + 40 c.c. 1.13 M Sugar.

- |  |  |
|--|--|
| (1) 1.2 c.c. M/60 tannic acid for 6 min. | . 30°/o eggs unchanged; some                                     |
| 1.3 c.c. N/10 ammonia for 1 hr.          | cytolysis and separation of<br>blastomeres; 10°/o blas-<br>tulæ. |
| (2) 1.4 c.c. M/60 tannic acid for 6 min. | . 40°/o blastulæ.  |
| 1.5 c.c. N/10 ammonia for 1 hr.          |  |
| (3) 1.6 c.c. M/60 tannic acid for 6 min. | . 20°/o blastulæ; great sepa-<br>ration of blastomeres.          |
| 1.7 c.c. N/10 ammonia for 1 hr.          |  |
| (4) 1.8 c.c. M/60 tannic acid for 6 min. | . 10°/o blastulæ; more cyto-<br>lysis than (1), (2), or (3).     |
| 1.9 c.c. N/10 ammonia for 1 hr.          |  |

Our final variation of Delage's method is described below :

(1) The eggs are placed in a solution of 100 c.c. sea-water + 40 c.c. 1.13 M. cane-sugar + 1.4 c.c. M/60 tannic acid for six minutes.

(2) 1.5 c.c. N/10 ammonia is added to the above solution and the eggs are left for one hour.

(3) The bowl is filled up with sea-water and the eggs are washed with three or four changes of sea-water before being finally left in normal sea-water.

At stage (3) the bowl has to be filled up with sea-water before the reagents can be drawn off, as the eggs float in the viscous sugary solution, but after adding sufficient sea-water they fall to the bottom. It is important to wash with three or four changes of water after the tannic acid, as traces of the latter left in the water cause a white precipitate to be thrown down. This precipitate only appears slowly, sometimes not for two or three days.



## On a Comparison of the above Experiments.

We have made many experiments, using both the methods described above on eggs from the same sea-urchin, to compare their relative efficiency for *E. esculentus* at Plymouth. We have always obtained a higher proportion of blastulæ by the use of tannic acid and ammonia, than by the use of hypertonic sea-water. Table XII gives two typical examples, one chosen to illustrate the comparative effects on a good batch of eggs, the other on a poor batch.

TABLE XII.

	Lot I.	Lot II.
(1) 3 c.c. N/10 butyric acid + 50 c.c. sea-water for 1.5 min.	60% blastulæ	Very few attempts at cleavage.
2 c.c. N/10 NaOH + 50 c.c. sea-water for 6 min.		
8 c.c. 2.5 M NaCl + 50 c.c. sea-water for 45 min.		Very irregular.
(2) 10 c.c. sea-water + 40 c.c. sugar + 1.4 c.c. M/60 tannic acid for 6 min.	80% blastulæ 10% eggs	40% regular blastulæ
1.5 c.c. N/10 ammonia added for 1 hr.	showing no change	Rest of eggs showing no change.

By the use of butyric acid and hypertonic sea-water 100 per cent. of the eggs are made to undergo some change, but the cultures always show much cytotoxicity and irregular cleavage. The blastulæ obtained by this method are healthy, they swim up to the top of the containing vessel in about 18 hours, and their mortality in the early stages is very low. With tannin and ammonia a higher percentage of normally shaped blastulæ is obtained, frequently 80 per cent. The eggs which do not give rise to blastulæ often do not show any change, and compared with the former method cytotoxicity is much less. The blastulæ, however, do not appear healthy, many never swim up to the top of the culture-vessel, and even at the four-armed pluteus stage may remain near the bottom of the jar. Their mortality for the first few days

is exceedingly high. There is no membrane formation by Delage's method, and this, together with the characters of the larvæ mentioned above, forms a parallel to the results obtained by Loeb (11) when using hypertonic sea-water alone as a method of starting segmentation.

#### V. COMBINED METHOD.

This suggested to us that possibly if the eggs of *E. esculentus* were treated, first with butyric acid to bring about membrane formation, and then with tannic acid and ammonia, we might succeed in rearing large numbers of healthy larvæ. This method, which is a combination of those elaborated by Loeb and Delage, has been the most successful at Plymouth this season (1912). Table XIII shows the relative proportions of blastulæ obtained by it, as compared with the numbers obtained by either of the other two methods.

TABLE XIII.

(1) 3 c.c. N/10 butyric acid + 50 c.c. sea-water for 1.5 min.	80 per cent. blastulæ swimming vigor- ously and very healthy in appear- ance.
10 c.c. sea-water + 40 c.c. sugar + 1.0 c.c. M/60 tannic acid for 6 min.	
1.1 c.c. N/10 ammonia added for 1 hour	
(2) 3 c.c. N/10 butyric acid + 50 c.c. sea-water for 1.5 min.	75 per cent. blastulæ.
10 c.c. sea-water + 40 c.c. sugar + 2.0 c.c. M/60 tannic acid for 6 min.	
2.1 c.c. N/10 ammonia added for 1 hour.	
(3) 10 c.c. sea-water + 40 c.c. sugar + 1.4 c.c. M/60 tannic acid for 6 min.	70 per cent. blastulæ.
1.5 c.c. N/10 ammonia for 1 hour.	
(4) 3 c.c. N/10 butyric acid + 50 c.c. sea-water for 1.5 min.	60 per cent. blastulæ.
2 c.c. N/10 NaOH + 50 c.c. sea-water for 6 min.	
8 c.c. 2.5 M NaCl + 50 c.c. sea-water for 45 min.	

The blastulæ obtained by this combined method are very healthy. They swim up to the top of the culture vessel within 18 hours, and up to the eight-armed pluteus stage grow more rapidly than normally fertilised eggs.

Given in detail the method consists in treating the eggs with—

(1) 3 c.c. N/10 butyric acid + 50 c.c. sea-water for 1·5 minutes.

(2) Wash in two or three changes of sea-water. Transfer to—

(3) 10 c.c. sea-water + 40 c.c. 1·13 M sucrose solution + 1·4 c.c. M/60 tannic acid for 6 minutes.

(4) 1·5 c.c. N/10 ammonia is added to (3) for 1 hour.

(5) Wash in three or four changes of sea-water, transfer to—

(6) Normal sea-water.

We have been unsuccessful, however, in bringing any of the larvæ obtained by this method through metamorphosis, despite the fact that they at first grew considerably faster than the normal sperm-fertilised ones. Their rapid rate of growth at first is remarkable, as the plutei obtained by this method have frequently attained the eight-armed condition in half the time that it takes the normal larvæ to reach this stage. Their development becomes, however, very slow after this, and only a few lived to develop an Echinus-rudiment.

The great objection to this method, and the probable reason why the larvæ obtained by it die off so rapidly in the late stages, is due to the difficulty of getting rid of all traces of the chemical solutions with which the eggs have been treated. When the eggs are transferred finally to normal sea-water, this in a few days' time, despite repeated washings, invariably goes cloudy and the larvæ seem to be killed. In some cases we washed the eggs in as many as thirty or forty changes of sea-water, and still the whole jar went cloudy in the course of a few days. We had recourse to pipetting off a few larvæ and placing them in a separate jar, as the only successful way of overcoming this difficulty. We were, however, unable to get any

of the few larvæ we separated in this manner to pass through metamorphosis, although they remained alive for several months.

This method would seem to offer considerable advantages over the other methods we have tried, if the difficulty of washing the eggs thoroughly could be properly overcome. It can hardly be said to have been given a fair trial by us this season, on account of so much of our material this year being unfavourable.

## VI. CONCLUSION AND SUMMARY.

Contrary to the experiences of Delage (5), we have always found that the parthenogenetic larvæ are readily distinguishable from the normal ones. This is most marked in the length of the arms and other minor features. Comparison of fig. 1, which represents a normal larva of *E. esculentus*, with that of fig. 8 brings out this point clearly. These two larvæ are of the same age approximately as regards development. That represented in fig. 1 is somewhat more advanced than that of fig. 8. The arms of the larvæ shown in fig. 8 are a third longer than those of fig. 1. The age of the larva of fig. 1 is twelve days, while that of fig. 8 is eight days. This illustrates the second point of difference between the two kinds of larvæ—their rate of growth.

In the later eight-armed condition, fig. 4, which represents a parthenogenetic larva twenty-five days old, this slender long condition of the arms is not so marked as in the earlier stage shown in fig. 8. At this stage the growth of the parthenogenetic larvæ is much slower than that of the normal ones. This is again brought out by a comparison of figs. 4 and 10. Fig. 4 represents a parthenogenetic larva twenty-five days old, while that of fig. 10 represents a normal larva twenty-two days old. It will be seen that the larva of fig. 10 is far in advance of that of fig. 4. The *Echinus*-rudiment in that of fig. 10 is well formed, while that of fig. 4, if present, is hardly distinguishable.

While the long slender arms of the parthenogenetic larvæ is the most obvious feature by which in the early stages they can be distinguished from the normal ones, a constant difference is also present in the arrangement of the pigment. In the normal larvæ this is more or less sharply localised in the tips of the arms, and in definite regions of the body, where it collects in dense masses. In the parthenogenetic larva this arrangement is much less definite, and the pigment seems more uniformly distributed throughout the arms and body. Again, all our parthenogenetic larvæ have shown a slight opacity of their cytoplasm, no matter by what methods they have been obtained. In the normal larvæ the cytoplasm is extremely clear and transparent, while in the parthenogenetic larvæ it never attains this excessively transparent condition.

Beyond these differences just enumerated, there seems no distinction between the parthenogenetic larvæ and the normal ones, and these differences, after all, are of minor importance. We have always been able to distinguish one from the other, however, throughout the course of our work.

#### SUMMARY.

This work was carried out during the seasons 1911 and 1912 at the laboratory of the Marine Biological Association, Plymouth. The experiments were made in order to obtain a method that could be applied to the sea-urchins of the English coast, and in the hope of raising some of the parthenogenetic larvæ to maturity.

We have been successful in rearing the plutei through the late stages, and large numbers in our cultures formed *Echinus*-rudiments. A few of the larvæ completed their metamorphosis, but we have not succeeded in getting the young urchins to live for more than a few weeks. In all about fifteen plutei underwent metamorphosis.

The methods used were those elaborated by Loeb and by Delage, and a new method which combined certain features from each of these.

Loeb's method consists in treating the unfertilised eggs with butyric acid to cause membrane-formation, and subsequently with hypertonic sea-water.

Owing to the condition of the sea-water at Plymouth it is necessary, after membrane formation, to place the eggs in water of raised OH ion concentration before treating them with hypertonic sea-water. The modification of Loeb's method which we employed is as follows. The unfertilised eggs were placed in—

- (1) 3 c.c. N/10 butyric acid + 50 c.c. sea-water for 1.5 minutes.
- (2) 0.2 c.c. N/10 NaOH + 50 c.c. sea-water for 6 minutes.
- (3) 8 c.c. 2.5 M/NaCl + 5 c.c. sea-water for .75–1 hour.

The best experiments with this method gave 60 per cent. blastulæ. The larvæ were healthy and grew well. All those plutei that underwent metamorphosis were obtained by this method.

Delage's method consists in treating the unfertilised eggs with tannic acid and ammonia in a mixture of sea-water and cane-sugar. The proportions we found most favourable for *E. esculentus* at Plymouth were 10 c.c. sea-water + 40 c.c. cane-sugar solution (strength, 388 grms. to a litre) + 1.4 c.c. M/60 tannic acid. The unfertilised eggs were placed in this for six minutes, and then 1.5 c.c. M/10 ammonia was added for one hour. We have obtained 80 per cent. blastulæ by this method, but they were not healthy, and died off rapidly during the first week. There is no membrane formation by this method.

The method which we have found most successful consisted in treating the unfertilised eggs first with butyric acid to cause membrane formation, as in Loeb's method, and then by Delage's method, as described above. In this way we have obtained as many as 90 per cent. blastulæ. The larvæ are vigorous, and grow for the first three weeks more rapidly than larvæ from fertilised eggs. We were, however, unable to get any of these larvæ to successfully metamorphose.

Given in detail, this method consists in treating the eggs as follows :

(1) 3 c.c. N/10 butyric acid + 50 c.c. sea-water for 1·5 minutes.

(2) Wash in two or three changes of sea-water and transfer to—

(3) 10 c.c. sea-water + 40 c.c. 1·13 M sucrose solution + 1·4 c.c. M/60 tanic acid for 6 minutes.

(4) 1·5 c.c. N/10 ammonia is added to (3) for 1 hour.

(5) Wash in three or four changes of sea-water and transfer to normal sea-water.

Finally, there is always a slight difference distinguishable between all parthenogenetic larvæ as compared with normal sperm-fertilised ones. This is noticeable in length of arms, pigmentation, and rate of growth.

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# EXPLANATION OF PLATES 30-32.

Illustrating the paper by Mr. Cresswell Shearer and Miss Dorothy Jordan Lloyd. "On Methods of Producing Artificial Parthenogenesis in *Echinus esculentus*, and the Rearing of the Parthenogenetic Plutei through Metamorphosis."

## LETTERING.

*a. cil. ep.* Anterior ciliated epaulette. *al. a.* Antero-lateral arm. *an.* Anus. *cel.* Celomic sac. *ech. r.* Echinus rudiment. *m.* Mouth. *p. cil. ep.* Posterior ciliated epaulette. *pd. a.* Postero-dorsal arm. *ped.* Pedicellaria. *po. a.* Postoral arm. *pro. a.* Preoral arm. *st.* Stomach.

## PLATE 30.

All the figures refer to *Echinus esculentus*.

Fig. 1.—Normal pluteus; dorsal aspect; 12 days old.  $\times 100$ .

Fig. 2.—Normal pluteus; 4 days; dorsal aspect.  $\times 100$ .

Fig. 3.—Parthenogenetic pluteus. Loeb's method  $MgCl_2$ ; 4 days. Highly abnormal; ventro-lateral aspect.  $\times 100$ .

Fig. 4.—Typical parthenogenetic pluteus; 25 days; dorsal aspect; posterior ciliated epaulettes beginning to be cut off. Loeb's improved method.  $\times 50$ .

Fig. 5.—Parthenogenetic pluteus; 4 days; left lateral aspect. Loeb's  $MgCl_2$  method.  $\times 100$ .

Fig. 6.—Parthenogenetic pluteus; 4 days; ventral aspect. Loeb's improved method.  $\times 100$ .

Fig. 7.—Parthenogenetic pluteus; 5 days; left lateral aspect. Loeb's method.  $\times 100$ .

Fig. 8.—Parthenogenetic pluteus; Typical; 8 days; dorsal aspect. Loeb's method.  $\times 50$ .

Fig. 9.—Parthenogenetic pluteus; 45 days old; ventral aspect.  $\times 100$ .

Fig. 10.—Normal pluteus; 22 days; dorsal aspect.  $\times 50$ .

## PLATE 31.

Fig. 11.—Young metamorphosed *Echinus*; ventral view; raised from egg fertilised with sperm.  $\times 100$ .

Fig. 12.—Parthenogenetic Echinus; dorsal aspect. Still retains portion of the pluteus skin, through which some of the spines are seen projecting dorsally.  $\times 100$ .

Fig. 13.—Young Echinus shortly after metamorphosis; ventral aspect. Raised from egg fertilised with sperm.  $\times 100$ .

Fig. 14.—Young parthenogenetic Echinus; ventral aspect. Loeb's method.  $\times 100$ .

Fig. 15.—Metamorphosing parthenogenetic Echinus. Loeb's method.  $\times 100$ .

Fig. 16.—Parthenogenetic pluteus showing Echinus-rudiment.  $\times 100$ .

### PLATE 32.

Fig. 17.—Parthenogenetic pluteus; 25 days; ventral aspect; Echinus-rudiment showing.  $\times 50$ .

Fig. 18.—Parthenogenetic pluteus; 26 days; ventro-lateral aspect.  $\times 50$ .

Fig. 19.—Parthenogenetic pluteus; 22 days; ventral aspect; showing Echinus-rudiment.  $\times 50$ .

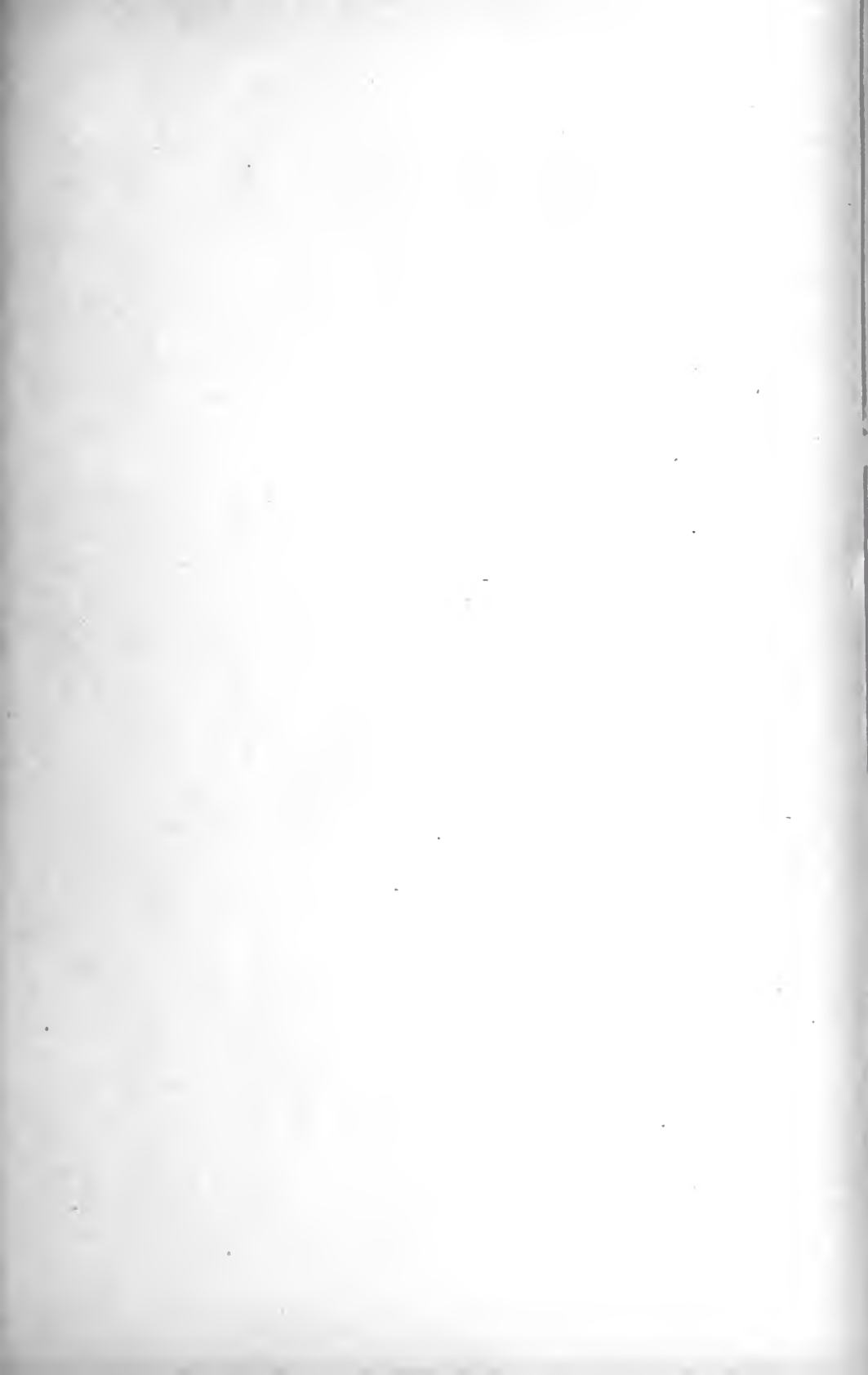
Fig. 20.—Parthenogenetic pluteus; dorsal aspect; 4 days.  $\times 50$ .

Fig. 21.—Parthenogenetic pluteus; dorso-lateral aspect; 5 days.  $\times 50$ .

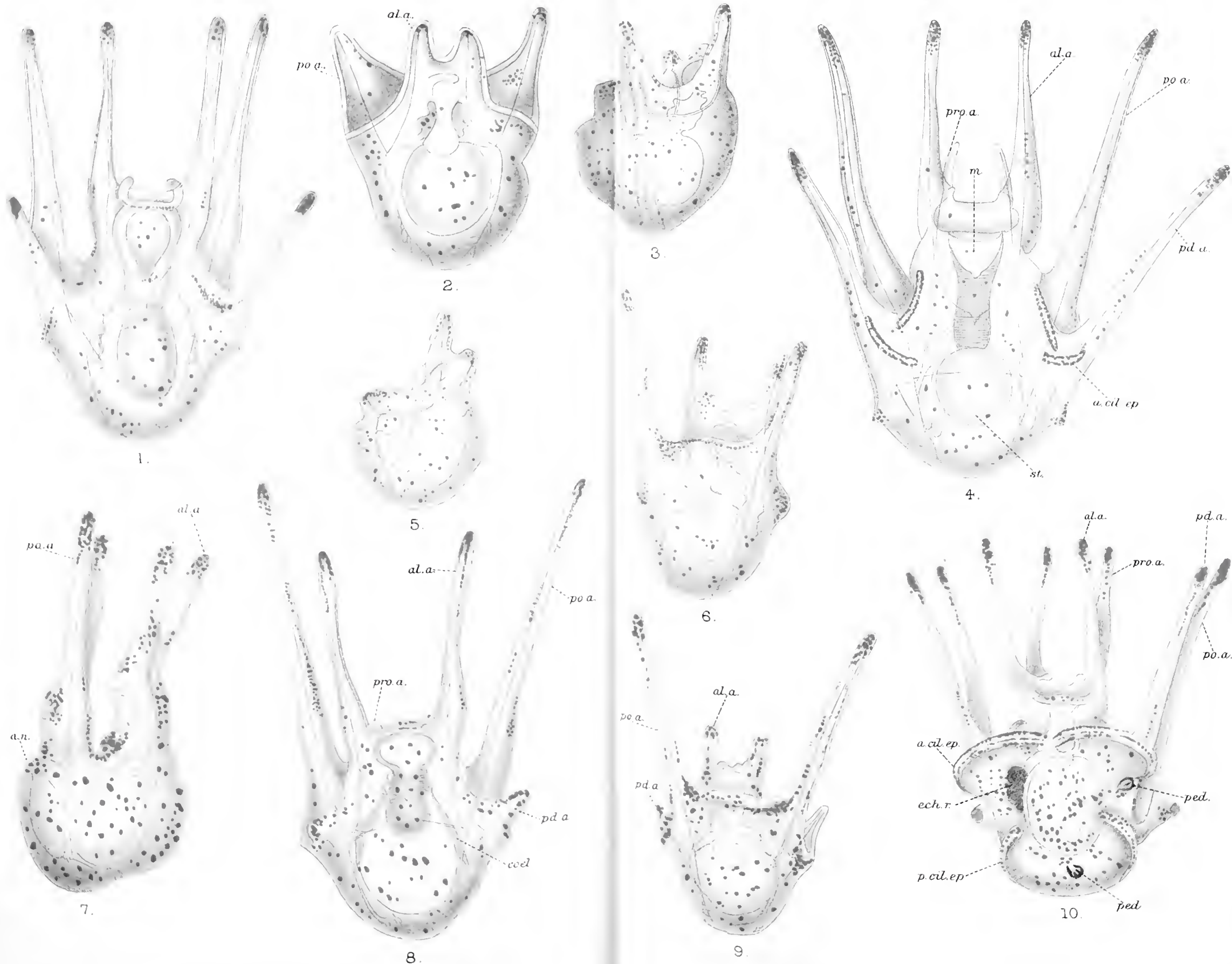
Fig. 22.—Parthenogenetic pluteus; 22 days; ventral aspect. A very abnormal form frequently turning up in culture-jars.  $\times 50$ .

Fig. 23.—Parthenogenetic pluteus; dorsal aspect; much degenerated. 22 days.  $\times 50$ .







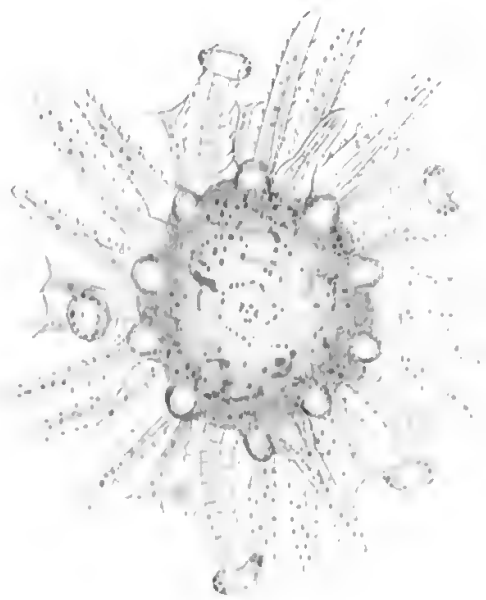




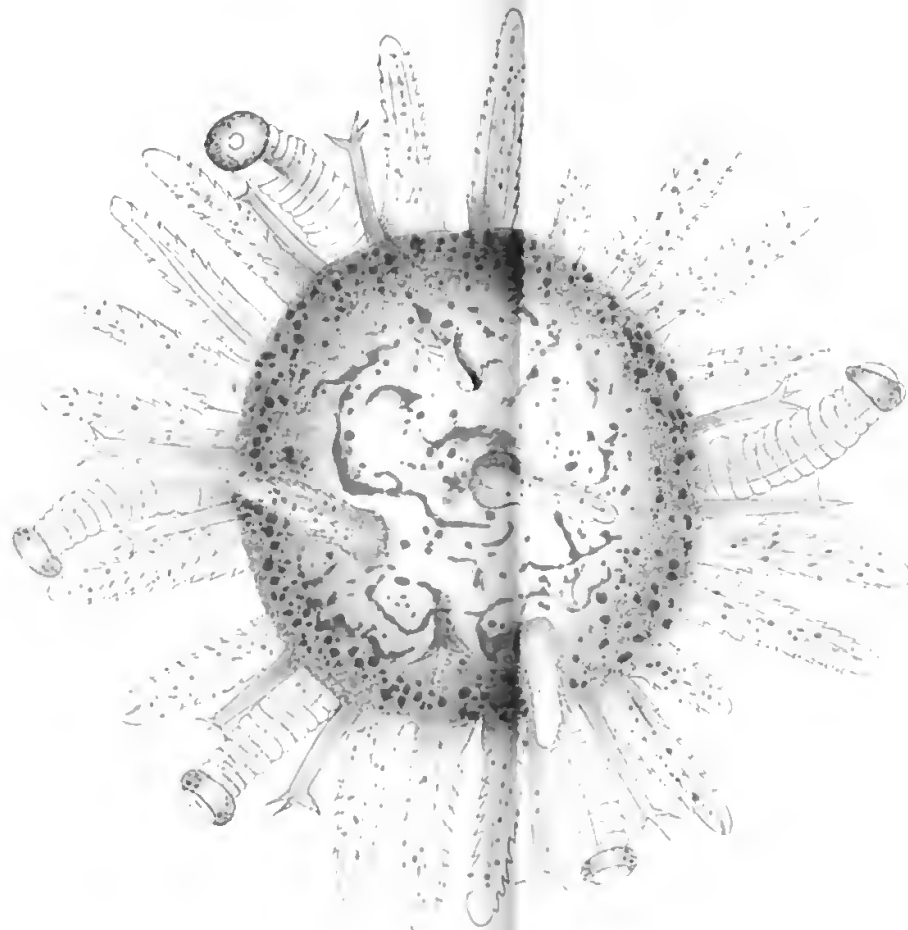




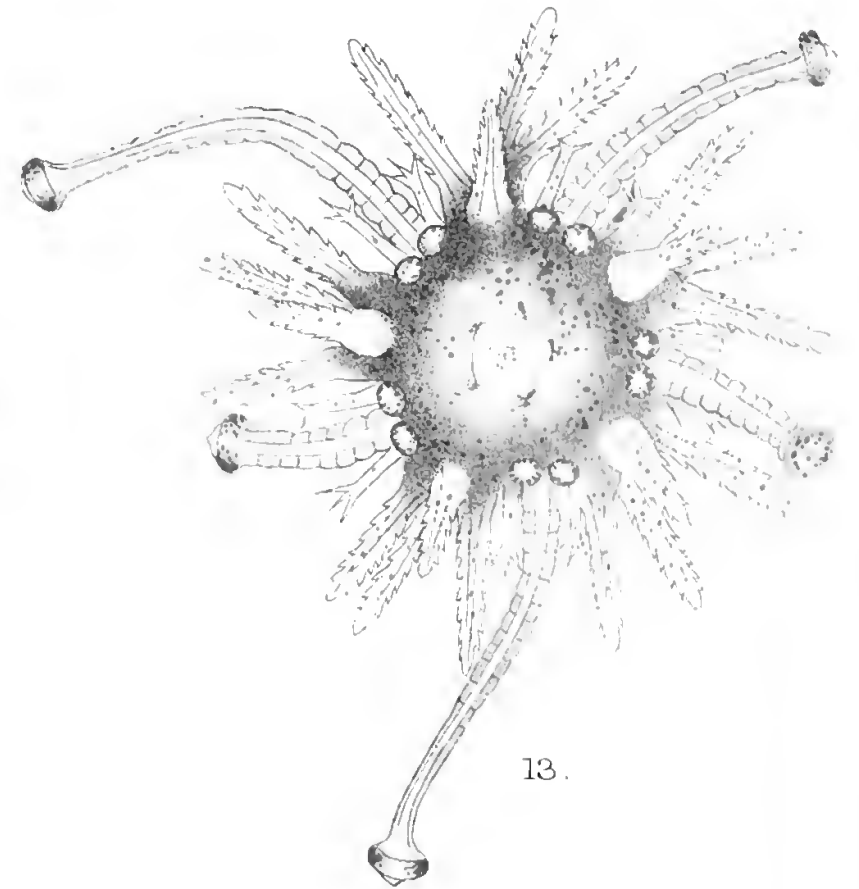




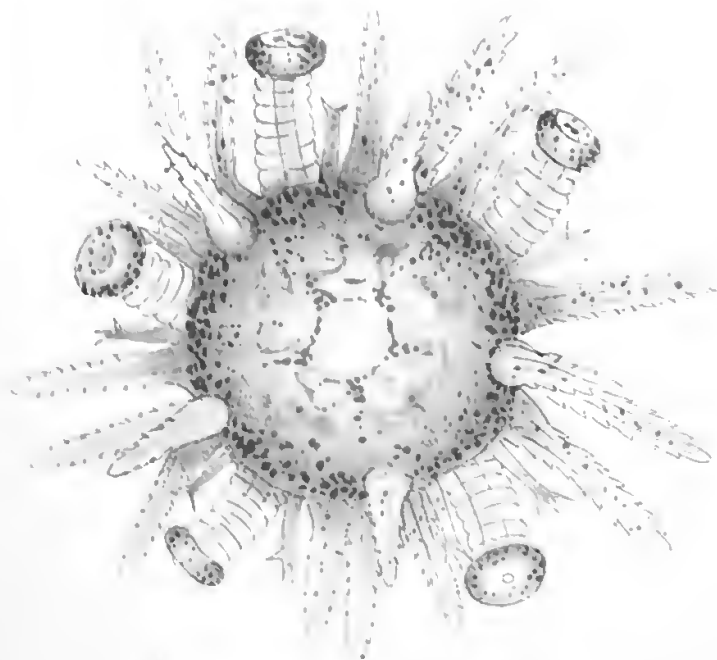
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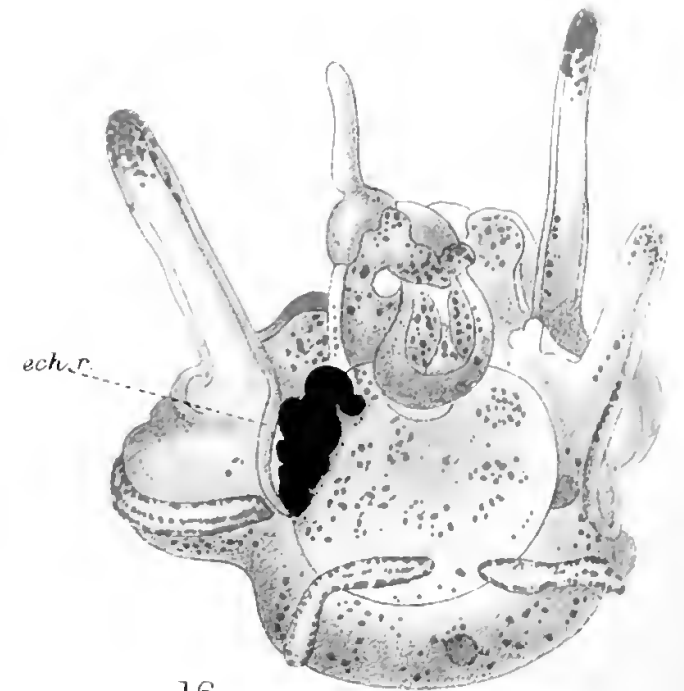
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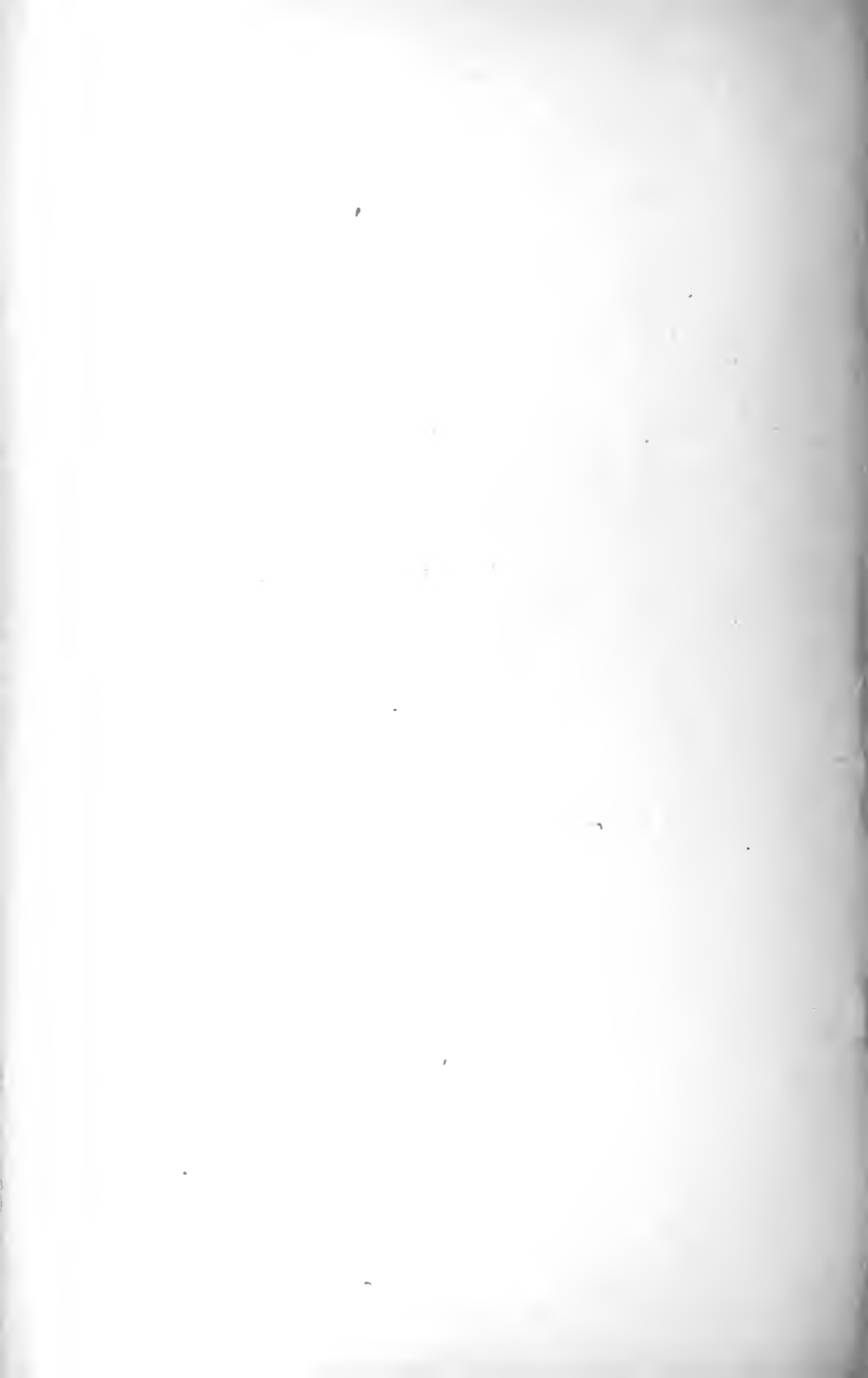
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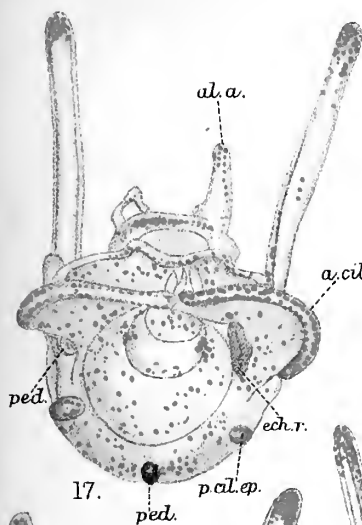


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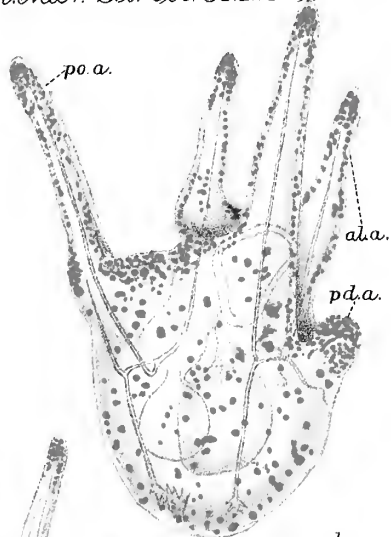


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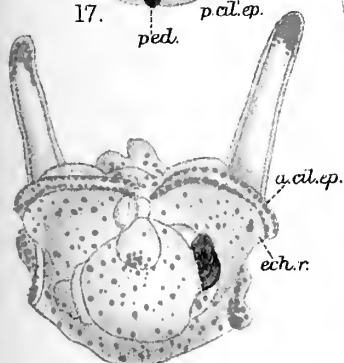




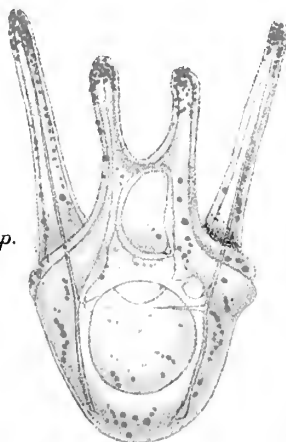
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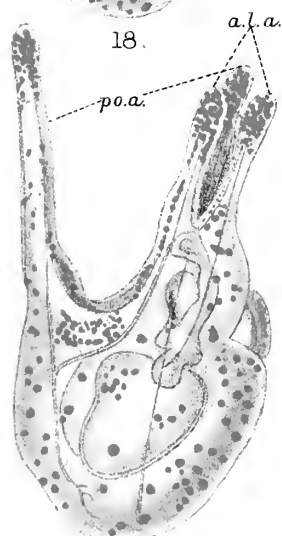
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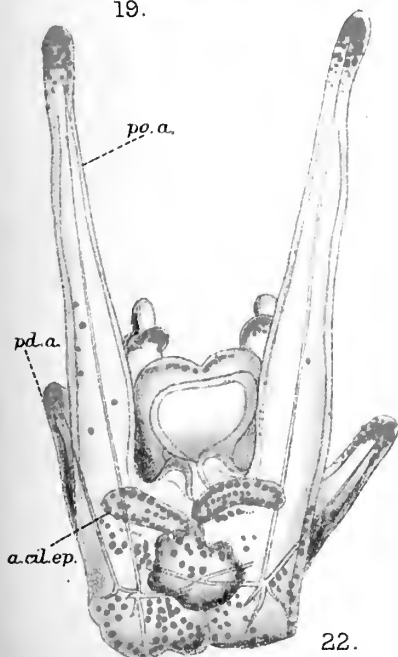
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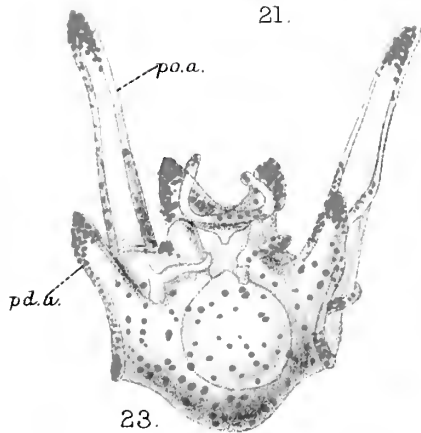
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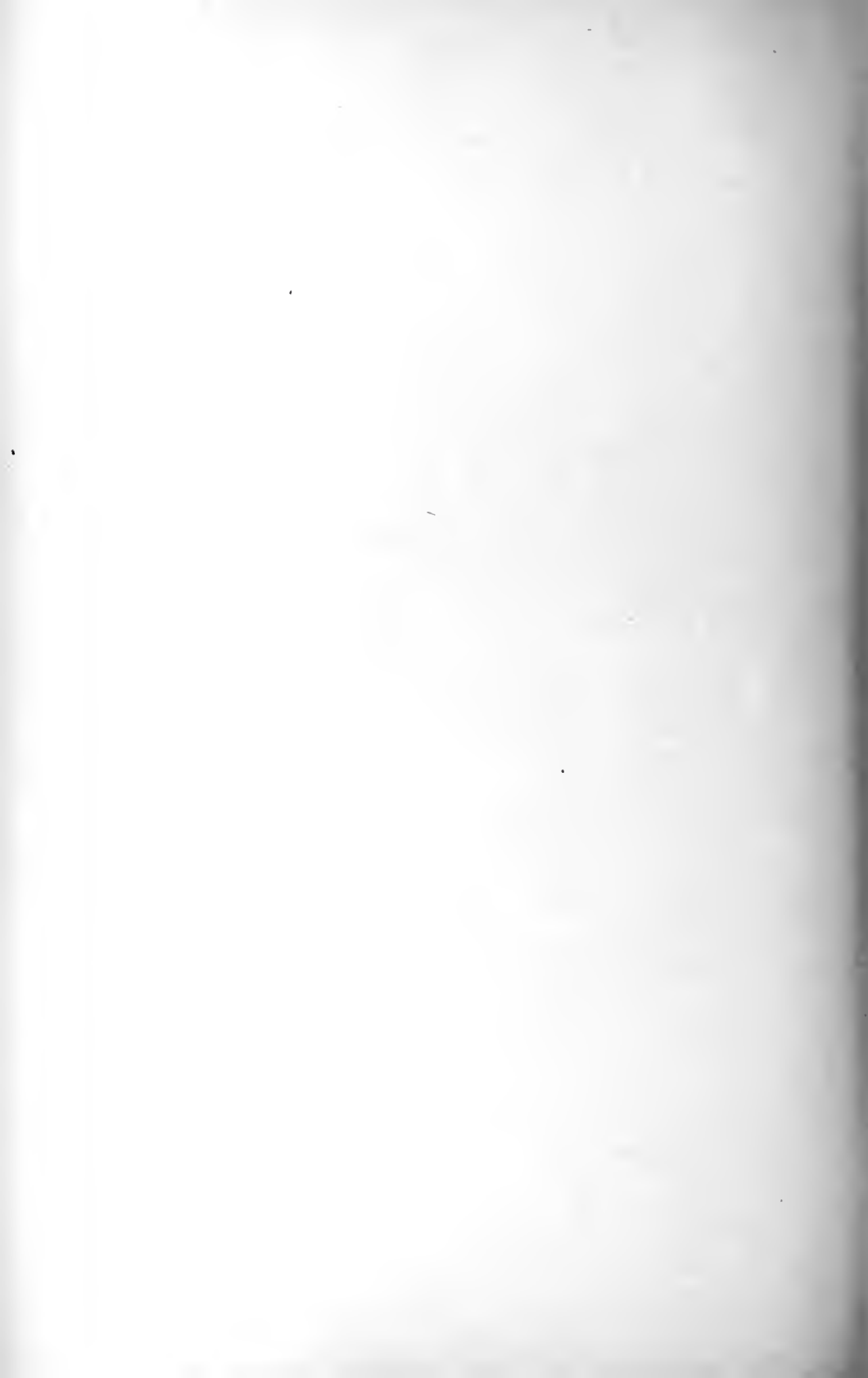
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## Changes in Chondriosomes Occurring in Pathological Conditions.

By

**J. O. Wakelin Barratt.**

From the Cancer Research Laboratory, University of Liverpool.

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With 11 Text-figures.

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THE structural characters of chondriosomes were first investigated in detail by Benda<sup>1</sup> in a number of different cell types (renal cells, spermatids, marrow cells, leucocytes, striated muscle).

Subsequently Meves,<sup>2</sup> by means of embryological observations, studied the metamorphoses of chondriosomes<sup>3</sup> in relation to differentiation of cell function, and showed that thereby different types of fibrils resulted, such as the cytoplasmic fibrils of epidermal cells, the fibrils of striped and unstriped muscle, neurofibrils, neuroglia fibres and connective-tissue fibres.

Although the structural characters of chondriosomes have been studied in detail, nevertheless the delimitation of mitochondria is in some cases still uncertain, and the full significance of chondriosomes in respect of cell function has yet to be determined. It has, however, been found possible in some cases, especially in respect of secreting cells, to form a

<sup>1</sup> Benda, "Weitere Mitteilungen über die Mitochondria," 'Verh. d. Phys. Ges. zu Berlin,' 1899.

<sup>2</sup> Meves, "Die Chondriosomen als Träger erblicher Anlagen, Cyto-logische Studien am Hühnerembryo," 'Arch. f. Mikr. Anat.,' 1908, B. 72, S.

<sup>3</sup> Mitochondria (*μίτρος*, a thread; *χόνειρος*, a grain) are granular; chondriokonts (*κοντός*, a pole) are rod-like. Chondriosome (*σῶμα*, a body) is a general term applied to both varieties. Illustrations are given in the figures.

conception of the significance of these granular or rod-like structures found in cytoplasm. Thus it has been shown by Regaud<sup>1</sup> that chondriosomes form the matrix of the secretory granules in the cells of the convoluted tubules of the kidney, and G. Arnold,<sup>2</sup> in an investigation upon the secretory activity of pancreatic cells, has traced the formation of zymogen granules by the maturation of chondriosomes.

Various theories have been advanced in explanation of the significance of chondriosomes in relation to cell functions. Benda regarded chondriosomes as representing a contractile constituent of the cell. Meves<sup>3</sup> has advanced the hypothesis that they are carriers of hereditary functions. More recently Regaud,<sup>4</sup> following Altmann and J. Arnold, has attributed to chondriosomes the function of fixing and concentrating various substances in the cell (*fonction électrique*, Renault)—an hypothesis which, as he observes, is not opposed to that of Meves, the two hypotheses rather representing different points of view. The conception of function advanced by Regaud is in complete harmony with the behaviour of chondriosomes in relation to secretion as exhibited by the cells of secretory glands.

Observation does not appear to have been up to the present directed to the study of chondriosomes in morbid cell states. Such investigation, however, appears likely to throw further light upon the significance of these cell structures.

In the present paper the condition of the chondriosomes is

<sup>1</sup> Regaud, C. L.—“Participation du chondriosome à la formation des grains de ségrégation dans les cellules des tubes contournés du rein (chez les ophiidiens et les amphibiens).” ‘*Compt. Rend. de la Soc. de Biologie*,’ 1909, t. 66, p. 1034.

<sup>2</sup> Arnold, G., “The rôle of the chondriosomes in the cells of the guinea-pig’s pancreas,” ‘*Arch. f. Zellforschung*,’ 1912, B. 8. S. 252. Cp. L. Launoy, “Contribution à l’étude histo-physiologique de la sécrétion pancréatique,” ‘*Arch. Internat. Phys. Liège*,’ 1905, vol. iii.

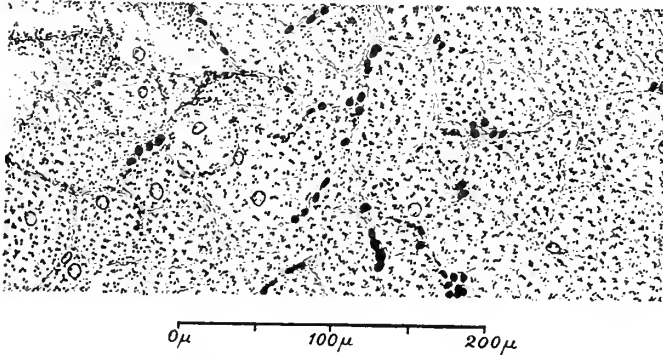
<sup>3</sup> Meves.—*Loc. cit.*

<sup>4</sup> Regaud, C. L.—“Sur la signification physiologique du chondriome des cellules sexuelles mures, notamment des spermatozoïdes,” ‘*Compt. Rend. de la Soc. de Biologie*,’ 1909, t. 66, p. 443.

investigated in respect of three pathological cell states, namely: (1) in that obtaining in the cells of the liver when pigmentary degeneration has occurred; (2) in that obtaining in the convoluted tubules of the kidney during severe hæmoglobinæmia; and (3) in the epidermis when a marked degree of epithelial proliferation has been set up. In all these cases examination of chondriosomes is readily effected, special methods of chondriosomal fixation not being required, and

FIG. 1.

[The scale of figs. 1, 2, 5 and 6 is given below fig. 1; that of figs. 3, 4, 7, 8, 9 and 10, below figs. 3 and 4.]



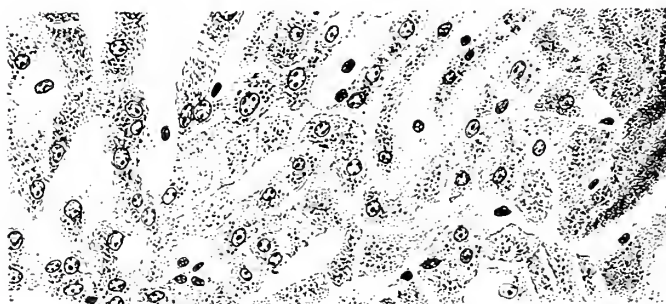
Section of liver of healthy rabbit. The liver-cells are closely apposed, the usual arrangement in cell columns being obscured. The blood-capillaries are indicated by red blood-cells darkly stained. The cytoplasm of the liver-cells exhibits numerous chondriosomes, appearing as deeply stained granules which obscure or conceal the nuclei. Fixed in Flemming's solution. Stained by Heidenhain's iron-alum hæmatoxylin method.  $\times 200$ .

thus the uncertainty and difficulty of chondriosomal staining is avoided. In the case of the liver and kidney it will be necessary, before studying the chondriosomes in the pathological state under consideration, to describe the normal appearance presented by the latter structures. It may be observed that in the liver and kidney, as in other secreting glands, the chondriosomes retain the embryonic type of

granule or rod throughout life, not undergoing any metamorphosis.

In the cells of the lobules of the liver of the adult rabbit fixed in Benda's or Flemming's solution, and stained by Heidenhain's iron-alum hæmatoxylin method (figs. 1 and 3), only mitochondria are normally met with. These are spherical or oval in shape, and are usually  $0.6\ \mu$  to  $1.0\ \mu$  in their greatest length, but the latter measurement may be exceeded, and, on the other hand, granules  $0.2\ \mu$  or less in diameter may be met with (fig. 3). The mitochondria are sharply outlined, and are

FIG. 2.



Section of liver of rabbit, exhibiting pigmentary degeneration. The liver-cells, which exhibit the normal arrangement in branching columns, contain dark pigment-granules, similar in size and arrangement to the chondriosomes shown in fig. 1, but less numerous. Fixed in Zenker's solution. Stained by Heidenhain's iron-alum hæmatoxylin method.  $\times 200$ .

scattered irregularly throughout the cytoplasm of the liver-cells, not being definitely arranged in chains or groups. They appear to vary in number. It is not, however, possible to determine accurately the number of chondriosomes present in a single cell owing to the difficulty of defining the exact limits of the hepatic cells. Nevertheless, seventy may be regarded as an approximate estimate of the number present in a single hepatic cell. The varieties observed in the chondriosomal content of healthy liver-cells in different rabbits (illustrated

by figs. 3 and 4), are apparently related to the functional or nutritive condition of the cell.<sup>1</sup>

The appearance of the pigment-granules (unstained) in the liver-cells in pigmentary degeneration (figs. 2 and 4) is, apart from their coloration, indistinguishable from that of the mitochondria (shown in fig. 3). The granules are spherical or oval in shape, and are scattered irregularly throughout the cytoplasm. Those shown in fig. 4 are  $0.3\mu$  to  $0.6\mu$  in length, but smaller ( $0.2\mu$ ) and larger ( $0.8\mu$ ) forms are also met with. The number of chondriosomes present in a single cell is

FIGS. 3 (to left) AND 4 (to right).

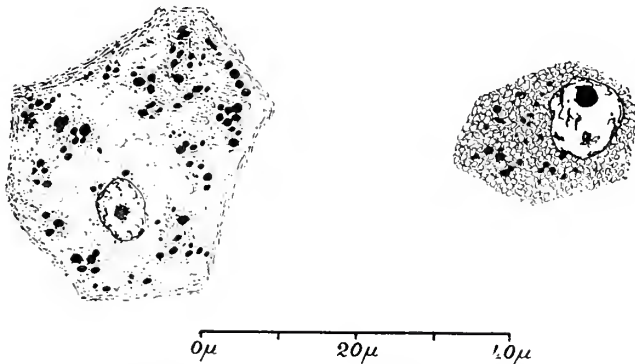


FIG. 3.—Liver-cell from fig. 1, more highly magnified. The chondriosomes, which are abundant, assume the form of well-defined, deeply stained granules, more or less ovoid in shape, ranging from  $0.5\mu$  to  $2.0\mu$  in diameter and arranged in groups. Fixed in Flemming's solution. Stained by Heidenhain's iron-alum hamatoxylin method.  $\times 1000$ .

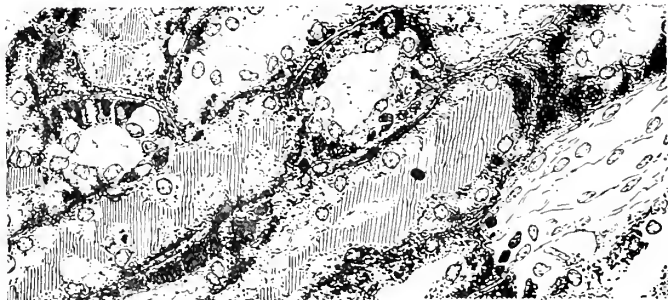
FIG. 4.—Liver-cell from fig. 2, more highly magnified. The cytoplasm is denser than in the cell shown in fig. 3 and exhibits a meshwork arrangement; it contains black (unstained) pigment-granules, which in appearance and size closely resemble the chondriosomes shown in the preceding figure, but are fewer in number. Fixed in Zenker's solution. Stained by Heidenhain's iron-alum hamatoxylin method.  $\times 1000$ .

<sup>1</sup> The rabbit from which figs. 2 and 4 were made was somewhat thin but seemed otherwise normal; its liver-cells were small in size, the cytoplasm being dense and presenting a finely vacuolated structure. The rabbit from which figs. 1 and 3 were made was exceedingly well nourished; its liver-cells were large, the cytoplasm being abundant.

approximately seventy, the variations met with not appearing to be considerable. The identity of the pigmented granules with mitochondria at once becomes obvious when a comparison of the two is made. The appearance of liver-cells, stained with hæmatoxylin, containing the former is indistinguishable from that of normal liver-cells similarly stained after treatment with mitochondrial fixatives (such as Benda's modification of Flemming's solution). In both cases the mitochondria appear of a deep black colour.

In sections of the kidney of healthy adult rabbits fixed in

FIG. 5.



Section of kidney of healthy rabbit at junction of cortex and medulla. The cells of the convoluted tubules shown in the section present a granular aspect, due to the presence in the cytoplasm of deeply stained chondriosomes, the nuclei tending to become obscured in consequence. A few red blood-cells, deeply stained, are seen lying between the tubules. In the lumen of three of the tubules hyaline material is seen. Fixed in Flemming's solution. Stained by Heidenhain's iron-alum hæmatoxylin method.  $\times 200$ .

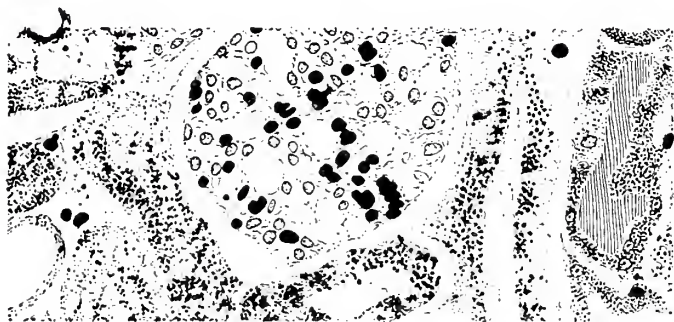
Benda's solution, and stained by Heidenhain's iron-alum hæmatoxylin method, the chondriosomes which are met with in the cells of the convoluted tubules<sup>1</sup> assume the form of rods and granules. In some of the cells only mitochondria are seen; in others—and this is more usually the case—chondriokonts are also met with, situated near the basement mem-

<sup>1</sup> I have not met with chondriosomes in the cells of the glomeruli.



brane, to which they are attached by one extremity, mitochondria being distributed throughout the cell elsewhere. In fig. 5 the appearance of the healthy kidney with the chondriosomes stained is shown under a low magnification, which, while showing indications of granules and collections of rod-like forms, does not permit of the arrangement of the chondriosomes being traced in detail. In fig. 7 a convoluted tubule cell taken from fig. 5 is shown under a higher magnification. Only mitochondria, it will be observed, are seen, but elsewhere in the same section cells conforming to the type

FIG. 6.



Section of cortex of kidney of rabbit during marked haemoglobinæmia. A glomerulus is seen, within the capillaries of which are red blood-cells deeply stained, but no granules are present. Several convoluted tubules are shown, the cells of which contain darkly stained granules, many of which are larger than those exhibited in fig. 5. The largest granules are disposed next to the lumen of the tubules. Hyaline material fills the lumen of the tubule lying to the right of the section. Fixed in Zenker's solution. Stained by Heidenhain's iron-alum hamatoxylin method.  $\times 200$ .

shown diagrammatically in fig. 9 are found. The mitochondria which are distributed throughout the cytoplasm resemble those already described, being sharply outlined, spherical or oval bodies, ranging from  $0.5\mu$  to  $0.8\mu$  in diameter; occasionally they reach  $1.0\mu$  in diameter; in other cases they do not measure more than  $0.2\mu$  across. The chondriokonts assume the form of short rods, sometimes very



fine, sometimes coarse, occasionally thicker at the end, and not infrequently presenting a flattened-out appearance. They measure  $0.5\ \mu$  to  $0.8\ \mu$  in thickness, and reach about  $4.5\ \mu$  in length. The number of chondriosomes in individual convoluted tubule cells could not be accurately determined, but appeared to be approximately about fifty.

In sections of the kidney of a rabbit suffering from severe hæmoglobinuria following injection of a large amount of hæmoglobin (obtained from rabbit's red blood-cells) (fig. 6), or of a dog suffering from hæmaturia due to piroplasmosis, the renal cells exhibited chondriosomes, readily stained by Heidenhain's iron-alum hæmatoxylin method, after the

FIGS. 7 (to left) AND 8 (to right).



FIG. 7.—Renal cell taken from fig. 5, more highly magnified. The cytoplasm contains deeply stained chondriosomes, assuming the form of more or less oval granules  $0.5\ \mu$  to  $2.0\ \mu$  in diameter. Near the basement membrane these granules are small; towards the free surface large granules are seen. Fixed in Flemming's solution. Stained by Heidenhain's iron-alum hæmatoxylin method.  $\times 1000$ .

FIG. 8.—Renal cell, taken from fig. 6, more highly magnified. Near the basement membrane, chondriosomes, deeply stained, of small size and more or less elongated, are seen in the cytoplasm; towards the free surface of the cell large chondriosomes, reaching as much as  $4\ \mu$  in length, are observed. (The large, darkly stained mass lying wholly within the nucleus is a nucleolus; below and to the right of this is a large chondriosome lying upon the edge of the nucleus.) Fixed in Zenker's solution. Stained by Heidenhain's iron-alum hæmatoxylin method.  $\times 1000$ .

use of fixatives such as Zenker's solution and formaline which do not permit of the staining of chondriosomes in the normal kidney of the rabbit. As was first described and

figured by Yorke,<sup>1</sup> the cells of the convoluted tubules in such conditions are filled with granules which may be of unusually large size and are recognisable under a low magnification (fig. 8). Rod-like forms may also be present, but owing to their small size are not well seen. The granules which are situated towards the lumen of the tubules are well defined, more or less rounded in aspect, and reach as much as  $2\ \mu$  to  $2.5\ \mu$  in diameter, granules of this size being fairly numerous, while in exceptional cases a diameter of  $6\ \mu$  may be reached; the smaller granules measure  $0.5\ \mu$  to  $0.8\ \mu$  across, the smallest granules being, however,  $0.2\ \mu$  or less in diameter. These granules are irregularly scattered and exhibit no definite

FIGS. 9 (to left) AND 10 (to right).



FIG. 9.—Type of normal cell of convoluted tubule prior to excretory activity. The chondriosomes consist of chondriokonts, lying near the basement membrane, and mitochondria, arising from the chondriokonts, and forming an outer granular layer reaching to the striated border of the cell.  $\times 1000$ .

FIG. 10.—Type of cell of convoluted tubule in severe hæmoglobinæmia. The condition of the cell is indicative of extreme secretory activity. The chondriokonts are much finer than in the preceding figure, while the mitochondria and secretory granules are numerous and of unusually large size.  $\times 1000$ .

grouping. The rod-like forms shown in fig. 8 are slender and are situated more deeply than the granules, one end being attached to the basement membrane; their thickness usually ranges from  $0.3\ \mu$  to  $0.4\ \mu$ , their length being about  $3\ \mu$ . The number of rods and granules observed in the cells of the convoluted tubules appeared to be approximately the same as in normal cells. In some cells no chondriokonts could be seen, only mitochondria being observed. The smallest

<sup>1</sup> Yorke, W., "The Passage of Hæmoglobin through the Kidneys," 'Annals of Tropical Medicine and Parasitology,' 1911, vol. 5, p. 401.

granules are in the unstained condition colourless ; the larger granules are brownish, the depth of colour being proportional to their size. The coloured granules stain less deeply with hæmatoxylin than the smallest granules.

The granules and rods just described are obviously chondriosomes, some of which are larger than in the normal condition. The identity of the forms seen in hæmoglobinæmia with chondriosomes is here more strikingly exhibited than is the case in the instance furnished by the pigmented liver of the rabbit, for we are dealing with two types, namely, rod-like forms and granules, and in both the normal and pathological condition the passage of rod-like forms into granules can be observed. For convenience of comparison of healthy and abnormal renal chondriosomes, a semi-diagrammatic representation of the two types is afforded by figs. 9 and 10.

Regaud<sup>1</sup> has shown that in Ophidia and Amphibia the secretory granules of the kidney, which are discharged into the lumen of the convoluted tubule, arise in relation with the mitochondria, and these in turn are formed from chondriokonts. This author figures the chondriokonts and secretory granules in the different stages of secretory activity of the renal cells, pointing out that the former are least numerous when the latter are most abundant and vice versâ. The cell shown in fig. 8 and represented diagrammatically in fig. 10 resembles an exaggerated degree of the condition figured by Regaud as that immediately preceding excretion, but it is not improbable that the functions of the cell represented in fig. 8 have in reality become disordered to such an extent as to imperil the integrity of the cell. The brownish colour of the larger granules during hæmoglobinæmia indicates the part taken by these structures in the elimination of hæmoglobin.

The significance of the pigmentary change exhibited by the cells of the liver is difficult to estimate, the relation of the chondriosomes to the secretory activity of these cells being

<sup>1</sup> Regaud.—*Loc. cit.*

unknown. It may, however, be observed that the functions of the hepatic cells in such cases do not seem to be seriously affected, for the condition is not incompatible with continued existence.

The chondriosomes of the epidermis have long been known as epidermal fibrils (*fibrilles epidermiques*, *Protoplasmafasern*), though their relation to chondriokonts has only recently been established by the researches of Firket.<sup>1</sup> So far back as 1899, Herxheimer<sup>2</sup> pointed out that these fibrils are most readily demonstrable in epithelioma, in warts, and in the apparently healthy skin in the neighbourhood of these lesions. Epidermal fibrils can also be exhibited with varying degrees of facility in callosities, at the edge of lupus areas and in chronic inflammatory conditions. In all these lesions, however, staining is more or less uncertain and is unequal in different parts of the same section. When overgrowth of epidermis has been produced in human skin or in the skin of the rabbit by means of the epidermal cell proliferant Scharlach R.<sup>3</sup> epidermal fibrils are much more easily exhibited, and the condition of the skin, both in respect of the extent to which cell hypertrophy takes place, and of the degree to which the chondriosomes become stained, can be modified by varying, on the one hand, the amount or concentration of Scharlach R introduced into the skin, and, on the other hand, by suitably choosing the period after injection at which the skin is sectioned, so that excellent preparations exhibiting the condition of the epidermal fibrils may in this way be obtained. The effect of the dye, like that of hæmoglobin in

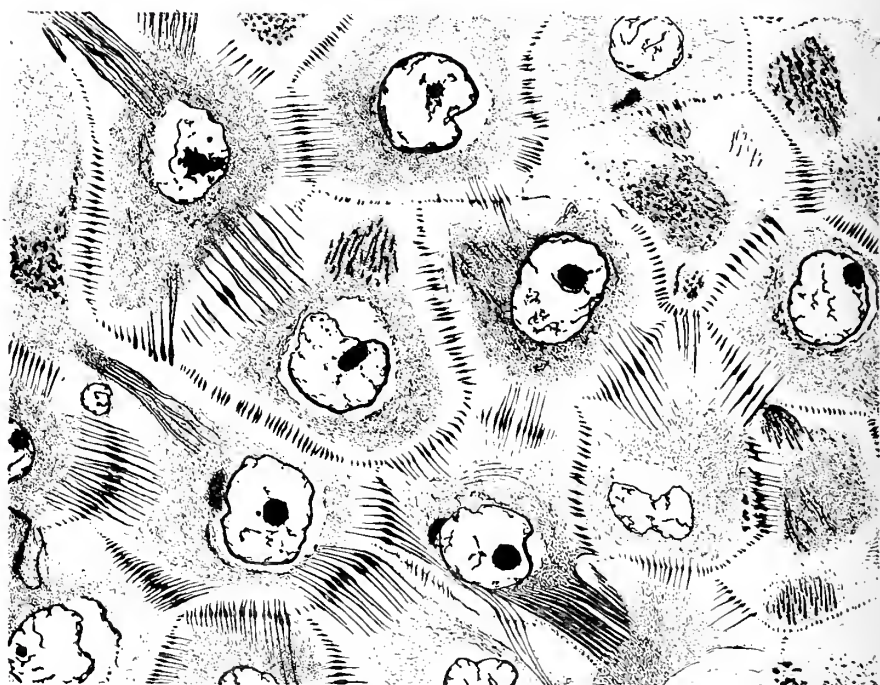
<sup>1</sup> J. Firket—"Recherches sur la g n se des fibrilles  pidermiques chez le poulet," 'Anat. Anz.,' 1911, Bd. xxxviii.

<sup>2</sup> K. Herxheimer.—" ber eigent mliche Fasern in der Epidermis und Epithel verschiedener Schleimh ute," 'Arch. f. Dermatol. u. Syph.,' 1889, Bd. xxi.

<sup>3</sup> B. Fischer.—"Die experimentelle Erzeugung atypischer Epithelwucherung und die Entstehung b sartige Geschw lste," 'M nch. med. Wochenschr.,' 1906, 53 Jahrg., S. 2041; J. O. Wakelin Barratt, "Implantation of actively proliferating Epithelium," 'Proc. Roy. Soc.,' 1907, ser. B., vol. lxxix, p. 546.

the case of the kidney, may in fact be compared to that of a mordant, enabling structures to be exhibited which would otherwise remain unstained or stain very imperfectly. The action of Scharlach R is, however, exerted only upon the prickly layer; in the stratum granulosum, stratum lucidum and stratum corneum the fibrils are not demonstrable, though

FIG. 11.



Section of rapidly proliferating Malpighian layer of human epidermis. Epidermal fibrils are seen passing from cell to cell with varying degrees of obliquity. At the junction of the fibrils of adjacent cells nodular thickenings are seen forming the "prickles" of the Malpighian cells. The fibrils, which are arranged in bundles, pursue a curved course through the protoplasm of the prickle-cells, passing round the nucleus and then passing out of the cell again. When viewed at right angles to their length the fibrils present a sheaf-like arrangement; if seen lying in the axis of vision the surfaces of the cells present a hairy aspect.  $\times 2200$ .

the nodular junctions of the fibrils passing between adjacent cells are still recognisable.

The appearance of the prickly layer of the epidermis when undergoing active proliferation is shown in fig. 11. The epidermal fibrils are seen to be arranged in bundles or sheath-like collections which pass from cell to cell. Within the cells the fibrils can be traced through the cytoplasm; they do not enter the nucleus, though frequently lying near the nuclear membrane as the figure illustrates. Although many of the fibrils entering the cell can be seen to pass out of the cell again, nevertheless the exact distribution of the fibrils in the prickly-layer is complex and difficult to follow out in its entirety. At the junction of the fibrils of adjacent cells a variable degree of thickening occurs, spindle-like nodules being thereby produced. When a number of such nodules are seen on the flat in optical section, as is shown in several cells in the upper half of fig. 11, a remarkable prickly or hirsute appearance is presented. The fibrils are of nearly uniform thickness throughout their course in the cell protoplasm.

The extent to which chondriosomes are altered in conditions involving active cell proliferation cannot be estimated with great precision owing to the impossibility of satisfactorily exhibiting the chondriosomes of the epidermis in perfectly healthy skin, for the methods of fixation and staining at present available usually reveal only the nodular thickenings at the points of junction of the fibrils of apposed cells, the fibrils themselves either remaining unstained—and this is the usual event—or staining imperfectly. As has been already mentioned, our knowledge of epidermal fibrils has been for the most part obtained by the study of the epidermis in pathological conditions. Judging by the appearance of the nodular junctions, it would seem that the chondriosomes in conditions of epithelial proliferation are markedly hypertrophied. Whether in addition any increase in the number of the fibrils or any change in their distribution in the cell protoplasm also occurs cannot at present be determined.

## Summary.

(1) The mitochondria of hepatic cells in pigmented degeneration of the liver assume a brownish-black colour and form the pigment-granules characteristic of this condition.

(2) In severe hæmoglobinæmia the chondriosomes of the cells of the convoluted tubules are more readily demonstrable than in the normal condition, their staining capacity being increased. In this condition the mitochondrial elements reach an abnormally large size and are observed to take part in the elimination of hæmoglobin.

(3) In pathological conditions in which rapid cell proliferation is occurring, the chondriosomes of the prickly layer of the epidermis appear of large size and stain with unusual facility, details of their structure being readily observable. In this respect they contrast with normal epidermal chondriosomes, which stain imperfectly.



## The Problem of Mitosis.

By

**C. F. U. Meek, M.Sc., F.L.S., F.Z.S.**

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### INTRODUCTION.

THE first comparison of the achromatic figure and that representing lines of force was made in 1873 by Fol, who likened the centrosomes to magnetic centres; and many theories of cell division have been based upon this similarity. Three years later Bütschli, who with Carnoy and van Beneden believed that the achromatic figure is formed anew at each mitosis, offered a definite explanation of division founded on his hypothesis of the alveolar constitution of protoplasm. According to this explanation diffusion currents flow towards the centrosomes, under whose influence the entire achromatic figure is evolved by morphological re-arrangement of protoplasm; and the resulting increase of surface tension at the equator causes the cell to divide. In 1878 Klein enunciated the rival theory of fibrillar contractility, based upon the reticular hypothesis of protoplasmic structure; he likewise believed that the achromatic figure is a re-arrangement of protoplasm under centrosome influence, but attributed the kinetic phenomena of division to contraction of fibrillæ. This theory was independently suggested by van Beneden in 1883. Later, Nägeli attempted to explain division by an assumed increase of the cell periphery; and Carnoy suggested that an emanation of ferments from the poles is the cause of aster formation.

In 1887 van Beneden elaborated the theory of fibrillar con-

tractility, assuming the spindle to be a differentiated portion of the asters, which were said to be radial arrangements of protoplasm. According to this theory the fission of the centrosome, or insertion point, causes the fibrillæ to become arranged in two radial and opposing groups, and their contraction results in division of the cell. This explanation was accepted in the following year by Boveri, who affirmed that in *Ascaris* the movement of chromosomes towards the equatorial plane, and the subsequent divergence of daughter-rods are caused by contraction of fibres that have become attached to them. Moreover, in 1889 Rabl upheld the view that the achromatic figure is only a re-arrangement of the resting-stage meshwork; whereas Boveri believed that it arises from the attraction sphere, or archoplasm, which was said to be composed of minute granules surrounding the centrosome and held there by its attractive force.

The first restriction of the contraction theory was made in 1891 by Hermann, who showed that the central spindle elongates during mitosis; he concluded from this that contractility in the spindle is confined to the mantle fibres, and suggested that the remainder are non-contractile supporting filaments forming a path for the chromosomes. The researches of Solger upon leucocytes, published at this time, seemed to corroborate van Beneden's theory; for their movements were attributed by him, and later by Heidenhain and Zimmermann, to contraction of permanent astral fibrillæ radiating throughout the cell. On the other hand, Bütschli in 1892 again affirmed that astral rays are not fibres, but lamellæ forming the walls of alveoli. In the same year Strasburger, who believed the entire achromatic figure to be derived from the cytoplasm, suggested that chromosome movement is due to chemotaxy; and Lustig and Galeotti showed that an unsymmetrical amphiasier may be the result of inequality of the centrosomes, and the cause of unequal distribution of chromosomes to daughter-cells.

In 1894 Heidenhain illustrated his theory of mitosis by a model, consisting of rubber bands placed at intervals round a

circular plate. According to this theory, which in principle resembles that of van Beneden, the astral rays are in a state of tension, their common insertion point being the centrosome, which plays a passive part in mitosis: on fission of the centrosome a new equilibrium is established by divergence of the poles and formation of a spindle between them; and contraction of the fibres causes not only divergence of daughter-chromosomes, but constriction of the cytoplasm itself.

The contraction theory was further restricted in 1895 by Drüner, who denied contractility in the astral rays, and thus extended Hermann's qualification to the entire achromatic figure. He affirmed, moreover, that the central spindle is not formed in the earliest stage, as Heidenhain, Hermann, Kostanecki, and others believed, but arises later through union of astral rays of the two poles. Although his denial of contractility in the astral rays was not accepted by all investigators, Boveri, Flemming, Heidenhain, Kostanecki, and Meves adopted the view that centrosome divergence is caused by growth of the central spindle; and this explanation seemed to account for the contortion of these rays during the metaphase, and accorded with infusorial mitoses in which centrosomes were said to be absent. In this and the following year R. Hertwig and Morgan independently produced asters in unfertilised Echinoderm ova by chemical treatment; and Schaudinn described a new formation of centrosomes in the swarm spores of *Acanthocystis*. Furthermore Boveri concluded from the study of abnormal Echinoderm ova that spindle formation is uninfluenced by the nucleus, and that cell division not only depends upon both asters and spindle, but cannot occur in the absence of chromatin.

The existence of elastic or contractile fibres was at this time denied by Gallardo, who formulated an electric interpretation of mitosis, assuming the centrosomes to be poles of opposite signs. This, however, was criticised by Meves, who said that crossing of rays is impossible in an electric field of unlike poles, and corroborated Drüner in restricting contractility to the mantle fibres. Rhumbler, on the other

hand, attributed cell division to the influence of a local thickening upon the surrounding cytoplasm.

In 1897 Erlanger put forward a theory similar in certain respects to that of Rhumbler. His researches upon Cephalopod cells and Echinoderm ova led him to believe that the aster, under centrosome influence, exercises a chemical or physical attraction upon the cytoplasm. He said that during the prophase of mitosis the nucleus absorbs fluid from the surrounding cytoplasm, and that its membrane disappears when a maximum volume is reached; fluid is then withdrawn by the asters, particularly from the nucleus, whose achromatic substance is thereby converted into a spindle; later the asters give up to the daughter-nuclei the fluid previously absorbed, and consequently diminish until they finally disappear in the resting stage. In the same year Kostanecki, who regarded the centrosome merely as an organ of insertion, suggested that the monocentric aster is converted into the spindle by longitudinal cleavage of its rays; but this has not been corroborated by subsequent investigation.

Following the researches of Mottier, Osterhaut, and Strasburger, who denied the presence of centrosomes in Pteridophyta and Phanerogams, R. Hertwig published in 1898 the result of his investigations upon *Actinosphaerium*. He believed that the central spindle is derived from the nucleus, and the asters from the cytoplasm; and affirmed that throughout the animal kingdom changes in form of the nucleus during mitosis represent a process of growth, and are caused by forces arising within it. Although denying that nuclear elongation is caused by contractile fibres, he did not exclude the possibility of contraction in mitosis; and thus agreed with Meves, who said that we must determine whether at a given moment a particular fibre causes movement by contraction or elongation. Moreover, Calkins and Ishikawa adopted the view that centrosome divergence is due to growth of the central spindle; and the former, who agreed with R. Hertwig regarding the origin of the metazoan centrosome, affirmed

that mantle fibres do not shorten during mitosis—thus contractility was denied to every portion of the achromatic figure in turn.

During the same year His remarked that elasticity of fibres is irreconcilable with the fluid condition of protoplasm: and showed that the contraction theory must at least be modified; for in trout germ-cells the mantle fibres are not attached by their ends to the chromosomes, but pass over them. He observed no congestion of thickened fibres in telophases, as must occur if contractile fibres are present, and affirmed that the problem of mitotic mechanism will not be solved until further analysis has been made of the chemical influences working in division. Moreover, Ziegler denied that mitosis in *Beroë* can be explained by contraction or elongation of fibres. He believed that the ovum consists of an inner vesicular yolk mass surrounded by a thin protoplasmic layer, and that cell division is caused by a thickening of the last-named, arising under centrosome influence in the region of the segmentation furrow. This idea of peripheral change caused by distant action of the centrosome was, however, criticised by Fischel and dismissed as purely hypothetical.

The crossing of rays was at this time dealt with by Bütschli and Rhumbler. The former showed that a figure resembling a mitotic spindle can be produced artificially if a warm film of gelatin containing air-bubbles is cooled and later coagulated with chromic acid. The latter pointed out that protoplasmic meshwork is held together by cohesive force, and that changes in form of alveoli must depend upon this as well as upon the forces that effect division: thus the crossing of rays was said to be impossible only if the centrosomes exercise an equal and uniform attraction upon an uniform cytoplasmic network. This explanation was accepted by Häcker.

In this year Meves published an exhaustive criticism of cell-division theories. Rejecting Rhumbler's explanation as inconclusive, he repeated that rays cannot represent lines of force because they cross, and pointed out that spindle

formation is impossible in an electric field of like poles; he believed that rays grow as new structures from the centrosomes, and thus disagreed with Bütschli, Erlanger, and Rhumbler, who regarded them as re-arrangements of pre-existing meshwork. He said that Ziegler's theory is untenable; and in this concurred with Rhumbler, who remarked that the distant centrosome action, postulated by Ziegler, can have no direction, and that the yolk mass must seriously diminish the influence of the centrosome upon distant portions of the membrane.

Two years later Rhumbler elaborated his theory of cell division. He suggested that the centrosome is a local solidification of the alveolar wall substance, and that the increased adhesion at this spot causes the formation of an attraction sphere by driving fluid contents towards parts of the cell where pressure is less: the migration of fluid particularly affects the reticular rays that extend from the centrosomes; and these, in giving up fluid, tend to shorten, thus exercising a tractive force that results in division. Later, Wilson accepted his explanation of the crossing of rays, and repeated that an electric interpretation of mitosis involving the assumption of unlike poles is inconsistent with the occurrence of tripolar mitotic figures. He agreed with Boveri, Carnoy, and Meves that amphiastral fibres are a new formation, but pointed out that the contraction theory postulates opposite functions of mantle and central spindle fibres, which appear to be similar in every respect.

At this time Reinke dealt with mitotic figures of unequal poles in *Salamandra* larvæ and *Echinodermata*. He showed that the half spindle on the side of the stronger radiation is more pointed than that on the side of the less, and that the equatorial plane is nearer the latter than the former; he therefore assumed force centres of unequal strength and opposite signs, and regarded the equatorial plane as being in equilibrium. Although explaining the crossing of rays by assumed unsimultaneous action of the centrosomes, he said that we know nothing concerning the nature of the mitotic

force, and that spindles may be produced otherwise than electrically. In a criticism of current theories he allowed that the assumption of unlike electric poles seems to be disproved by tripolar mitotic figures; and showed that, since such poles will attract and not repel one another, a further assumption is necessary, e. g. that the centrosomes irritate the living substance between them, thus causing the growth of a spindle that pushes them apart. On the other hand, he regarded the smallness of the centrosomes as a serious objection to Bütschli's explanation of mitosis.

In 1903 Rhumbler published detailed studies of resemblance of mitotic figures and electric lines of force, and again denied that the former can be traced to the latter. He reiterated his theory of division, affirming that it not only accords with the occurrence of tripolar mitoses, but is the more compatible with cytological phenomena. At the same time Ziegler adhered to the view that cell division results from a change in the protoplasmic outer layer caused by centrosome action.

In 1905 Hartog said that division is caused by a "mitokinetic" force resembling magnetism; that protoplasmic components vary in their respective degrees of permeability to this force; and that the most permeable in the cytoplasm become converted in mitosis into "material chains" of force, differing from geometrical lines of force in that they can cross and anastomose. The chromosomes were likewise regarded as susceptible to induction, and, since contiguous portions presumably carry like charges, cleavage of the spireme and subsequent divergence of daughter-rods in mitosis were attributed to mutual repulsion. He believed that cytoplasmic traction is the cause of centrosome divergence, and pointed out that figures of Kostanecki and Yatsu prove that this divergence is not always observed during mitosis. In support of his theory he produced several magnetic figures, including a triaster obtained with two unlike poles and a third centre at zero. He rejected the contraction theory, as held by Boveri, firstly, because contractility must be confined to the



mantle fibres, and secondly, because in certain plants the chromosomes move along the fibres, and no contraction is observed; moreover, he affirmed that fibres, although flexible, afford no evidence of the pushing force assumed by Meves. Dismissing Bütschli's gelatin figures and Rhumbler's model as inconclusive, he denied the validity of their theories on the grounds that they involve spindle formation between like poles, and based this argument upon the work of Vejdovsky and Mrazek, who showed that the action of osmosis, currents, and surface tension in the cell is the same at both centres.

During the following year Gallardo put forward a modification of his earlier electric theory, based upon certain experiments of Lillie. The latter, after defining protoplasm as a complex aggregate of water and colloid and crystalloid substances, showed that free nuclei and spermatozooids follow the negative current when placed in a magnetic field; whereas cells rich in cytoplasm tend to follow the positive. He accordingly assumed that chromatin and cytoplasm carry electric charges of opposite signs, and pointed out that this assumption is consistent with their staining reactions. Gallardo thereupon assumed electric charges of opposite signs for the chromatin and cytoplasm in mitosis, and said that centrosomes move apart as a result of repulsion of like forces, while chromosome divergence is due to the combined effects of mutual repulsion and centrosome attraction. He explained cell division by assuming that the divergence of daughter-nuclei causes a fall of potential at the equator, thereby entailing in this region an increase of superficial tension and the formation of a zone of constriction. He accounted for the growth and fission of chromatin granules on the spireme by adopting Perrin's theory of segmentation and divergence: each granule was said to grow under the favourable influence of superficial tension and cohesion until sufficiently large to be inducted with electric charges of like sign; these act as an internal cause of dislocation, and by mutual repulsion effect fission of the granule, whose daughters undergo further

growth. He believed that the objection of crossed rays is removed by Hartog's distinction of material chains and theoretical lines of force, and said that not only had tripolar figures been produced magnetically, but that his theory is consistent with their occurrence in mitosis. He allowed, however, that charges of opposite signs must be assumed for the centrosomes of spindles that carry no chromosomes.

Two years later Baltzer published the results of his investigations upon multipolar mitoses in Echinoderm ova. He pointed out that, if centrosomes carry charges of unlike signs, Hartog's explanation of chromosome divergence fails; for each daughter-rod must be inducted by the near pole more strongly than by the farther, and the presence of unlike charges in sister rods will consequently cause attraction and not repulsion. Moreover, he repeated that the assumption of unlike poles cannot be reconciled with the occurrence of either typical triasters or tetrasters that have diagonal spindles. He remarked that Gallardo's qualification in the case of chromosomeless spindles involves opposite explanations for apparently similar data, and is incompatible with the occurrence of mitotic triasters in which two spindles containing chromosomes are united to one that contains none. Furthermore, he showed an early stage of an Echinoderm triaster in which three distinct spindles are formed while the chromosomes are still lying in the centre of the figure: and affirmed that the early stage of every mitosis is represented by a chromosomeless spindle; for the last named is stretched between the two poles for some time before the chromosomes take up their positions upon it. In criticising the conclusions of Reinke he pointed out that in Echinoderm mitosis the chromosomes can be equally distributed to the two poles when these are obviously unequal, and suggested that abnormal distribution is due to insufficient plane surface offered by the smaller aster. He remarked, in conclusion, that Rhumbler's explanation seems to be the most probable, and accords with multipolar mitoses and the occurrence of asters of various sizes.

In 1909 Gallardo replied to these criticisms. He withdrew his qualification in the case of chromosomeless spindles; and, instead, denied that they can be formed, affirming that their appearance is an illusion produced by juxtaposition of rays of two centrosomes lying close together. He saw no reason for regarding as chromosomeless the spindles in Baltzer's early stage triaster, and believed that these were formed in the normal manner and in accordance with his theory.

During the same year Hartog also published a reply to Baltzer's criticisms. Again postulating the dual character of the mitotic force, which was said to resemble electrostatic force or magnetism, he repeated that the centrosomes are unlike poles, and said that magnetic experiments suffice to remove Baltzer's objections. He pointed out that a triaster can be produced with a neutral iron core and two unlike magnetic poles, and that three charcoal iron balls, placed in a magnetic dust-field traversed by an electric current, behave as three equal and equidistant centres united by spindles. Furthermore, he showed that tetrasters having one diagonal spindle can be obtained with two unlike magnetic poles alternating with iron cores, and denied the existence of tetrasters with two diagonal spindles, as shown by Baltzer. In the following year he said that Gallardo's theory is improved, because cases are known in which full-grown spindles have been formed in the absence of chromatin, and agreed with Baltzer that in the animal cell the spindle is always at first chromosomeless. Since no spindle can be formed between like poles, and since the mitotic spindle appears to be homopolar with respect to all known forces, he concluded that mitokinetism is a new force unknown so far outside the living cell.

The views of Gallardo and Hartog were criticised by Baltzer in 1911. He pointed out that the denial of the existence of chromosomeless spindles is refuted by his figures and those given by Wilson in 1901; for in these the asters are too far apart to permit the explanation of juxtaposition: moreover, in Gallardo's figure explaining the early stage of the triaster, the lines of force are bent at the equator; and,

since this is not seen in the mitotic triaster, correspondence is incomplete. In dealing with Hartog's interpretation he repeated that the theory of unlike poles is disproved by triasters; for, in the figures obtained with the iron core, one spindle must always be formed more strongly than the other two, and an equilateral triaster, such as occurs in Echinoderm ova, can therefore never be obtained. He showed, moreover, that the figure produced by the charcoal iron balls differs from mitotic triasters in that spindle axes are straight in the latter and bent in the former. Furthermore, he pointed out that in magnetically produced tetrasters having one diagonal spindle, the last-named must lie in the long diagonal, i.e. between the two real poles; whereas in mitotic tetrasters it lies in the short diagonal, as is shown in his figures and those of O. and R. Hertwig. He then described two tetrasters with two diagonal spindles, obtained from sections stained with hæmatoxylin and light green, and superior to his older whole preparation; and thus again contradicted Hartog's denial of the existence of such figures in mitosis. With regard to contraction theories, he remarked that we know nothing concerning the nature of the rays; and said that the crossing of spindles, as observed in tetrasters, is explicable on the assumption that rays are either independent fibres or mutually dependent portions of a figure representing lines of force, because crossed rays have been found by Rhumbler not to lie in the same plane.

Closely following these criticisms, a paper appeared by Lawson upon the achromatic figure in vascular plants. In this he said that changes undergone by the chromatin during the prophase of mitosis cause the karyolymph gradually to diffuse by exosmosis through the nuclear membrane; and this diffusion is said to be the direct cause of diminution observed in the nuclear vacuole. He believed that the corresponding increase in volume of the cytoplasmic portion of the cell cavity involves a change of tension resulting in readjustment of the reticular structure, and that portions of the last-named immediately surrounding the nuclear vacuole are drawn out

in fine threads by the membrane as it contracts. When the karyolymph has completely passed into the cytoplasm the nuclear membrane is seen to have drawn the chromosomes into a compact group, and the membrane then envelops each chromosome, which thus receives an independent osmotic system. Moreover, since the contracting membrane draws after it a portion of the cytoplasmic reticulum in the form of fibres, each chromosome at this time is furnished with numerous fibres attached to the membrane enveloping it; and, since the fibres are said merely to represent lines of tension, each chromosome is compelled to assume a position such that its major axis is parallel to the equatorial plane. On arrival at the poles each daughter-chromosome becomes vacuolated by endosmosis; and the formation of a nuclear vacuole in each daughter-cell is regarded as an inverse repetition of the process seen in the prophase, in that karyolymph flows back from the cytoplasm through the membrane enveloping the chromosomes, thereby causing this membrane to expand. He attributed the apparent destruction of the nuclear membrane in preparations to the action of fixing reagents, which kill the cytoplasm; and pointed out that in the living cell we have no reason to suppose that it breaks down, and that, if it does break down, a new membrane must at once be precipitated by the cytoplasm. Thus the achromatic figure is regarded as a passive result of the movements in mitosis, and the fibres as the expression of lines of tension. The fibres neither grow out and attach themselves to the chromosomes, nor exercise a tractive force upon the latter; and the apparent movement of fibres, as seen in the convergence of cones, is merely an illusion produced by local alterations of tension entailing transference of expression from one locality to another.

At this time an explanation of cell division was put forward by Robertson. He said that, in the polar synthesis of nuclein from lecithin, cholin is formed as a bye-product; this was supposed to diffuse in all directions, and, reaching a maximum concentration at the equator, to cause decrease of surface

tension and consequent constriction of the cell. This, however, has been denied by McClendon, who pointed out that no appreciable synthesis of nuclein occurs during cleavage, and that Masing and Shackell have found as much nuclein in the two-cell stage of the egg as in the blastula. Moreover, he described an experiment showing that the surface tension is not diminished, but relatively increased.

The electric interpretation of mitosis was advocated last year by Pentimalli, who, with Gallardo, assumes a negative charge for the chromatin. In his experiments, which resemble those of Lillie, he showed that the chromosomes move towards the positive pole when an electric current is passed through the cell; and, when the cell membrane is perforated, free themselves from spindle and cytoplasm and pass into neighbouring cells. He affirmed that the spindle is not similarly affected, but seems to be drawn by the chromosome movement; and suggested that the electric charge of the chromatin increases as mitosis proceeds. His results have since been corroborated by McClendon.

Gallardo has recently published a paper summarising his views and offering a further explanation concerning chromosomeless spindles. He again points out that the theory of unlike poles cannot account for either the divergence of centrosomes in the prophase or that of daughter chromosomes in the anaphase; that it is impossible on this assumption to produce tetrasters with two diagonal spindles, or to produce triasters without having recourse to a neutral centre; and that the staining reactions at the two poles appear to be inconsistent with the theory. He affirms that these difficulties are removed by assuming the poles to be of the same sign, and says that all chromosomeless spindles are formed by the stretching of the viscous and elastic cytoplasmic medium.

#### DISCUSSION.

Consideration of the various theories outlined on the preceding pages and of the objections that have been raised against them shows that no unanimity of opinion exists



concerning this problem: in the circumstances we must try to discover if any of these theories constitute an adequate explanation.

We will first deal with the arguments of Hartog. He says: "The absolute proof of the opposite polarity of the two centrosomes is to be found in the growth of the spindle by inflexion and coalescence of rays growing out from the centrosomes." Now this can only mean that the mitotic spindle is formed by a force acting at two points, viz. its poles; and that, since a known force cannot produce a spindle figure between two points if these points represent like poles, the mitotic spindle must be heteropolar if it is produced by a known force. Let us assume that the spindle is formed in the manner described, and therefore that the conclusion is valid. Now he says: "Since it is demonstrated that the cell-spindle is homopolar with respect to osmosis, currents, electrolytic or electrostatic force, magnetism is out of the question, (and) we may conclude that mitokinetism is a new force unknown so far outside the living cell." By the first part of this sentence I understand that the mitotic spindle cannot be heteropolar with respect to known forces, because, if it is heteropolar, one point in certain mitotic figures must represent two poles of opposite signs—a *reductio ad absurdum*. By the second part I understand that, since it has already been shown that the spindle must be heteropolar if formed by known forces, such forces cannot be the cause of its existence; consequently we are dealing here with a new force. Let us consider this force. Now, if the spindle is formed as he believes by the action of this force at its poles, the last-named must be either unlike or like. We will assume the former. In this case certain mitotic figures cannot be explained unless we assume one pole to be of two opposite signs: and this is inconceivable. Let us now consider the alternative. In this case the new force is able to produce a spindle figure between two points at which it is given expression, when these points represent like poles. But this is contrary to fundamental principles of physical



science, and its acceptance necessitates the assumption that in the universe two sets of physical principles exist, diametrically opposed to one another. Which of these alternatives does Hartog ask us to accept? He asks us to accept the first; for he says that the visible structures of the polar stars and spindle are "material chains of force of more permeable substance than the rest of the field, held in position by the stresses radiating from the unlike poles." But this alternative has been shown to imply the inconceivable; and, since an explanation implies something that can be conceived, the assumption of his new force cannot explain spindle formation.

We must now ask if this new force can be the cause of the mitotic processes that result in the separation of daughter-bodies into two groups. We find that it cannot cause the divergence of either the centrosomes in the prophase, or the daughter-chromosomes in the anaphase. In the case of the former the unlike signs postulated must entail attraction and not repulsion: and Hartog himself has realised this; for in order to explain centrosome divergence he has been forced to assume "a bodily pull exercised by the cytoplasm."<sup>1</sup> In the case of the latter the daughter-chromosomes must presumably become inducted more strongly by the near than by the farther pole, and since the presence of unlike charges can cause only attraction, other forces must again be invoked to explain this divergence. But if essential processes of mitosis cannot be explained without invoking forces to counteract the effect of the new mitokinetic force, how can this latter force be put forward as the cause of mitotic phenomena? It cannot be so put forward. And, since the new force postulated by Hartog can neither cause the formation of the spindle, nor effect the processes for which mitosis has been instituted, his entire theory is found to be inadequate.

Before, however, leaving the subject let us look again into

<sup>1</sup> Since writing this I have received a communication from Prof. Hartog, who says that he now believes that centrosome divergence is caused by like osmotic and electric charges, which counteract the heteropolar mitokinetic force.

the arguments from which he has concluded that the spindle is formed by a new force. We find at once that the conclusion rests upon a belief that the spindle is formed by a force acting only at its poles, and, since the reasoning is logically valid and the other premises appear to be irrefutable, we must either accept the conclusion or reject the interpretation of spindle formation. Now we have already found that the action of a new force between unlike poles is inconceivable in certain figures; we must therefore choose between a method of spindle formation different from that assumed and the acceptance of a new force acting between like poles. But we have also found that the latter involves the further assumption that in the universe two sets of physical principles exist, diametrically opposed to one another. Consequently the new force must be regarded as a special creation in the narrowest sense of the words. We know that special creation has been the refuge of the baffled investigator throughout the history of scientific thought, and that it has seldom or never proved itself to have been justified: and in the circumstances I prefer to think that the spindle is not formed by a force acting only at its poles, rather than that it is the visible expression of a force whose action violates the principles upon which physical science is at present based.

Let us now turn to Gallardo's interpretation. This assumes that the centrosomes and chromosomes in mitosis carry respectively positive and negative electric charges; that the spindle, as seen in the metaphase, is composed of two spindles, each formed between a centrosome and the chromatin of the equatorial plate; and that cell division is a bipolar phenomenon, primarily electro-colloidal in nature. Let us consider first the assumption that the chromosomes carry negative electric charges. This was suggested by certain experiments of Lillie, who found that free nuclei and spermatozooids move towards the anode when placed in an electric field; moreover, Pentimalli, corroborated by McClendon, has since shown that the chromosomes move towards this pole when an electric current is passed through the cell. We know that particles

in colloid suspensions invariably carry charges of this sign, and, even if chromosomes cannot be regarded as such particles, the staining reaction of chromatin suggests at least the possibility of this potential. In the circumstances Gallardo seems to be justified in saying that this assumption rests upon a sound experimental basis: and, if found to be true, it may explain the divergence of daughter-chromosomes in the anaphase; for like charges must cause mutual repulsion. We will now consider the assumption that the centrosomes carry positive electric charges. This likewise was suggested by the experiments of Lillie, but only in so far as the cytoplasm as a whole was said to become inducted. Gallardo, however, says, "*Les centrosomes sont susceptibles d'acquérir un potentiel positif plus élevé que le cytoplasma, qui contient des microsomes d'un potentiel plus bas.*" But I see no proof of this: and Gallardo himself realises that it is hypothetical; for he says almost immediately afterwards, "*Le potentiel positif du centrosome augmente pour des causes inconnues et détermine sa bipartition et la séparation des deux centrosome fils entourés de radiations.*" And the second half of this sentence clearly shows his object in making the assumption. We will, however, suppose that the centrosomes represent definite positive poles, and will pass on to his interpretation of spindle formation.

By regarding the spindle of the metaphase as being composed of two half spindles, each formed between a centrosome and the chromatin of the equatorial plate, he is able to explain all mitotic figures containing chromosomes without asking us to imagine spindle formation between like poles. But the interpretation fails the moment that we consider chromosomeless spindles; and Gallardo has repeatedly tried to remove this objection. Firstly, he proposed charges of opposite signs for the centrosomes of these spindles; but the possibility of this was disproved by Baltzer, who pointed out that it is irreconcilable with the formation of triasters in which two spindles containing chromosomes are united to one that contains none. Gallardo thereupon fell back upon

a denial of the existence of all chromosomeless spindles, affirming that their appearance is an illusion. Baltzer, however, produced figures that cannot be thus explained, and said that the same conclusion must be drawn in the case of other spindles independently shown by Wilson; moreover, he pointed out that in the animal cell every spindle is at first chromosomeless. Gallardo now admits the existence of such spindles, and says, "Une difficulté se présente pour expliquer le petit fuseau primaire pendant l'éloignement des centrosomes; mais on peut admettre avec Enriques (1911) que ce fuseau est formé par l'étirement du milieu visqueux du cytoplasma. Cette même viscosité et élasticité du milieu cytoplasmique peuvent expliquer les rares cas connus de fuseaux sans chromosomes." Let us consider this explanation. Now, if the form of the spindle is controlled, as he affirms, by forces other than the electric charges at the poles, all the forces concerned either can or cannot be represented by resultants at these points. We will suppose that they can be so represented. In this case a spindle cannot be formed between like poles, nor can an anti-spindle be converted into a spindle without at the same time changing the signs of the poles from like to unlike. But, if the signs are thus changed, the theory of spindle formation is at once disproved by Baltzer's triaster in which two spindles carrying chromosomes are united to one that is chromosomeless; moreover, if the poles of the primary spindle are unlike, other forces must be invoked to explain their divergence, because unlike charges cannot cause repulsion. We are therefore compelled to assume the alternative, viz. that the action of the various forces concerned in spindle formation cannot be represented by resultants at the poles: and, if this is so, the spindle can no longer be regarded as a figure formed entirely by forces acting at the centrosomes; consequently the conclusion at which we arrived in our criticism of Hartog's theory appears to be justified.

We must now ask how far this conclusion affects Gallardo's interpretation of cell division; for the conformation of the

achromatic figure can now be no index of the actions at the poles. He says, "Les deux nouveaux noyaux en formation attirent le cytoplasma positif et déterminent ainsi la segmentation cellulaire. Le contour extérieur de la cellule suit dans cette segmentation le forme des équipotentiellles successives entre deux centres homonymes. L'approximation des deux nouveaux noyaux en formation aux centrosomes respectifs, de charge de nom contraire, produit une coagulation entre colloïdes de signes opposés (formation de nouvelles membranes nucléaires) et une neutralisation qui détermine une période d'équilibre." From this it is evident that the interpretation depends upon electric effects between the negatively charged chromatin and the positively charged centrosomes, and that the spindle itself is not actively concerned in these processes. But, although the assumption of a negative electric charge for the chromosomes has been shown to rest upon an experimental basis, the assumption that the centrosomes have a positive potential higher than that of other microsomes is hypothetical; and upon the latter assumption as well as upon the former rests his interpretation of both spindle formation and cell division. His proposition relating to bipolarity in the mitotic process is therefore entirely speculative so far as the centrosomes are concerned; and we must remember that, even if these are eventually found to be definite positive poles, electricity may not be the primary factor in division, and, in any case, the theory will require modification before it can explain the mitosis of those plants in which centrosomes do not exist.

We will now consider Rhumbler's theory. As already stated, the centrosome is said to arise as a local solidification of the alveolar wall substance; the solidification involves increased adhesion at this spot, and fluid is accordingly driven towards portions of the cell where pressure is less. The consequent loss of fluid in the reticular rays causes them to shorten, and thus exercise a tractive force that results in division. That the theory has been carefully thought out is evident; for it postulates a concatenation of events in mitosis,

and throughout its construction Rhumbler has kept before him the phenomena that he seeks to explain. But Hartog has pointed out that the actions of osmosis and currents in the cell have been shown to be the same at both poles, and that, because the two centrosomes must accordingly have the same sign, spindle formation between them is impossible. We must therefore ask if Rhumbler assumes the spindle to be a figure formed entirely by the action of forces at its poles; for, if he does not assume this, Hartog's objection need not be considered. Now, it is obvious that he does not regard the spindle as being formed in this manner, because, in order to overcome the difficulty presented by an assumed spindle formation between like poles, he invokes other forces, which are said to convert the anti-spindle into the spindle by causing coalescence of rays. This explanation is identical in principle with that put forward by Gallardo, and accords with the conclusion at which we arrived in our criticism of Hartog's theory.

We must now ask if Rhumbler's theory of mitosis depends for its proof upon the conformation of the spindle. It does depend upon it, because division is directly attributed to tractive forces exercised by the reticular rays. But, if the conformation of the achromatic figure can no longer be regarded as an index of the actions at the poles—and in the circumstances it cannot be regarded as an index—we have no reason for believing that the rays represent a migration of fluid from the region of the centrosomes. And, if proof of this migration is not established, we cannot infer that the rays contract as a result of loss of fluid. The theory therefore remains for the present a suggestion.

Let us now turn to the recent work of Lawson. This is not offered as an explanation of mitosis, but merely constitutes an interpretation of the achromatic figure in vascular plants. His researches have led him to affirm that in the prophase the karyolymph diffuses through the nuclear membrane into the cytoplasm, thus causing diminution of the nuclear vacuole; that the contracting membrane draws the



bivalent chromosomes into a compact group at the equator ; that the membrane never breaks down, but envelops each chromosome in the metaphase and anaphase ; and lastly, that the spindle-cones are fibrils drawn out from the cytoplasmic reticulum by the receding membrane, and attached at one extremity to the chromosome envelopes. He concludes from these data that the cones are a visible expression of tension, and believes that the divergence of cone apices merely represents transference of tension from one locality to another and is unaccompanied by movement of the fibres themselves. His first two assertions, which are the result of direct observation, constitute a simple and efficacious method of collecting the scattered chromosomes into one spot before division, and may be the correct explanation of this phenomenon. His next assertion, however, is entirely hypothetical. Firstly, he asks us to believe in the existence of a membrane at a time when he himself says that it is invisible. Secondly, his drawings are inconclusive. He says that fig. 22, pl. 48, shows the beginning of envelopment of individual chromosomes by the membrane : but in this figure I see only a slightly lobulate appearance of the nucleus such as is often observed in somatic and spermatogonial resting stages. His fourth assertion is equivalent to saying that the spindle-cones are composed of mantle fibres ; for he affirms that the fibres are attached at one extremity to the chromosome envelopes. But, since the belief in the existence of these envelopes rests upon no evidence, this assertion also must be regarded as hypothetical.

Before, however, passing on, let us suppose that his data are correct, and ask how far his conclusions, which concern only mantle fibres, affect spindle interpretation in other types. In the animal kingdom we find asters and a primary spindle, upon which the chromosomes later become arranged and divide : the presence of this spindle can no longer be denied ; for considerable controversy has recently taken place concerning it, and Gallardo himself has now been forced to admit its existence. If we turn to Meves' paper upon the



spermatogenesis of *Salamandra maculosa* (1896), we see in fig. 55, pl. 4, a typical example of this spindle and its asters; and the arrangement of the mantle fibres closely resembles that of the cones drawn by Lawson. Now Lawson regards the cones as a visible expression of tension, and suggests that this tension "decreases in proportion with the distance from the nuclear membrane." And, since the primary spindle, as is shown in Meves' figure, lies between centrosomes placed at the apices of the cones, it cannot have been formed entirely by the action of forces expressed by the cones; for the forces postulated by Lawson, however attenuated at these points, are like. But, if the spindle is not formed entirely by the action of forces expressed in the cones, to what is its formation due? Lawson's interpretation does not profess to tell us, and cannot tell us. It is therefore unnecessary for us to discuss it further.

We will now consider the older theories of mitosis. These may be classified as centrosome theories and fibre theories. Let us deal first with centrosome theories. Meves defined these by saying: "Unter Centrentheorien verstehe ich solche, welche die bei der Mitose wirksamen Kräfte in die an den Spindelpolen befindlichen Gebilde verlegen und die Strahlungen als die erscheinende Wirkung dieser Kräfte ansehen." He pointed out that all such interpretations of spindle formation necessitate the assumption of either unlike or like poles; and said in the former case that rays cannot cross, and in the latter that no spindle can be produced. With respect to his first objection, the adherents of these theories affirm that we are dealing here with material chains, and not theoretical lines of force, and that crossing of rays is therefore possible. We need not, however, consider this possibility; for we have found that the assumption of unlike poles is irreconcilable with the occurrence of certain mitotic figures, and this alone suffices to disprove the interpretation. With respect to his objection to the assumption of like poles, we have already shown in our criticism of Gallardo's theory that, if the forces concerned in spindle formation can be expressed by resultants

at the poles, it is impossible to convert the anti-spindle into the spindle without at the same time changing the signs of the poles from like to unlike; and that, if the forces cannot be so expressed, the spindle is not a figure formed entirely by the action of forces at its poles. In the circumstances we must accept Meves' conclusion that: "Die Strahlungen nicht als die sichtbar werdende Wirkung einer in den Centren lokalisierten Kraft anzusehen sind; womit aber nicht ausgeschlossen ist dass die Centren bezw. Centrankörper überhaupt Einflüsse irgend welcher Art auf das umgehende Cytoplasma ausüben." And, since the conformation of the spindle figure can no longer be regarded as an index of the actions at the poles, all early theories that depend for proof upon this conformation must now become mere hypotheses.

Meves defined theories belonging to the second class by saying: "Fadentheorien sind solche, nach welchen die wirkenden Kräfte ausschliesslich in den Fäden ihren Sitz haben und nach welchen die im Centrum der Strahlungen gelegenen Gebilde in erster Linie die Rolle von Insertionsmittelpunkten besitzen." They were, moreover, divided into two sub-classes according as the kinetic phenomena of mitosis were attributed entirely to contraction or to contraction and elongation of the rays. Such were the theories of van Beneden, Boveri, Drüner, Flemming, Hermann, O. and R. Hertwig, Meves, Rabl, Solger, and Zimmermann. We have seen in the introduction of this paper that objections have been raised against these theories, and that independent investigators have denied contractility in every portion of the achromatic figure in turn. In the circumstances the theories belonging to the first sub-class seem to be completely disproved; and in view of the conflicting evidence those belonging to the second must be regarded as speculative in that they assume that the mantle fibres pull the daughter-chromosomes apart.

#### CONCLUSION.

Let us now consider our position. We have found that none of the theories discussed can be accepted as an adequate

explanation of mitosis; for those that are not actually disproved appear to be either partly or entirely hypothetical. In the circumstances there is only one course of action left for us to adopt. We must try to discover if there is any generalisation upon which all these theories are in agreement, or that may be regarded as having been established by the reasonings of their supporters. It is at once evident that there is such a generalisation, viz. that the mitotic spindle is not a figure formed entirely by the action of forces at its poles. Hartog's arguments have proved that, if this is denied, the spindle cannot be formed by known forces. Gallardo and Rhumbler have been forced to accept this proposition; although the latter, in doing so, has sacrificed the proof upon which he sought to establish his theory. Lawson's interpretation of mantle-fibre formation involves this proposition; and we have seen that the older theories either admit it, or are disproved if they do not admit it. This, therefore, and this alone, is the basis upon which we must raise the superstructure that will eventually explain these phenomena.

Is there a second generalisation that can be regarded as established? There is none; for all others are either hypothetical or dependent upon personal observations that are not accepted by all investigators. There is, however, one conclusion that must be drawn from the proposition that we have established, viz. that the spindle figure cannot now be regarded as an index of the actions at the poles: at present we have no means of determining how far these actions, if existing, are limited or counteracted by the other forces whose presence we have been compelled to admit; and, until this is known, no theory depending entirely for proof upon the conformation of the spindle figure can be regarded as other than hypothetical. For this reason the utility of models in the immediate future seems to be doubtful.

In the circumstances we must collect data, and not attempt to furnish an explanation of mitosis until we have been able

to establish further generalisations upon which investigators are agreed. Unfortunately the technical difficulties of this research and the small likelihood of obtaining results leading directly to an explanation have deterred students from undertaking investigations: the work done has consequently been confined to the laboratories of a few eminent cytologists. Only by patiently collecting data and building one established proposition upon another shall we arrive at the solution of this problem, which we have found to be so complex.

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**On the Afferent Ganglionated Nerve-fibres of  
the Muscles Innervated by the Fifth Cranial  
Nerve; and on the Innervation of the Tensor  
Veli Palatini and Tensor Tympani.**

By

**F. H. Edgeworth, M.D.,**

Professor of Medicine, University of Bristol.

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With Plates 33 to 36.

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It was shown by Sherrington that the skeletal muscles innervated by the spinal nerves receive afferent nerve-fibres from the posterior nerve-roots as well as efferent nerve-fibres from the anterior roots. These afferent nerve-fibres come from cells in the spinal root ganglia, and constitute from one third to one half of the myelinate fibres in any muscular nerve-trunk. On the other hand, the external ocular muscles do not receive any ganglionated nerve-fibres, and the direct fibres which pass to them are efferent-afferent (Sherrington, Sherrington and Tozer, Dogiel).

It is of interest to inquire whether the muscles innervated by the fifth cranial nerve resemble the skeletal muscles innervated by the spinal nerves in receiving ganglionated afferent nerve-fibres, or whether they resemble the external ocular muscles in not receiving such nerve-fibres.

The subject has already been investigated, with very diverse conclusions.

Sappey ('72) stated that Palleta, Louth and Longet, "ne voient dans cette union"—of the motor and sensory parts of the mandibular division of the fifth cranial nerve—"qu'un

simple accollement et admettent en conséquence que le nerf maxillaire inférieur se compose de deux branches parfaitement distinctes dans toute l'étendue de leur distribution, une branche inférieure et interne ou sensitive, et une branche supérieure et externe ou motrice, qui a reçu tour à tour les noms de nerf buccinato-buccal, de nerf masticateur, de nerf maxillaire inférieur moteur."

Sappey himself was of a different opinion. He held that "les deux branches du nerf maxillaire inférieur s'envoient réciproquement un grand nombre de filets"; that "parmi les divisions de ce tronc nerveux s'il en est qui se détachent plus particulièrement de la racine motrice, et d'autres de la racine sensitive, les premières renferment aussi quelques fibres destinées à des organes sensibles, et les secondes quelques fibres destinées à des muscles."

His ('87) made the following statements: "Als am meisten dem Typus am Rückenmarksnerven folgend, sieht man bekanntlich den Trigemini an. Hier verfolgt man die motorische Wurzel, an den G. Gasseri vorbei, bis zu ihrer Verbindung mit dem Aste der sensibeln Anlage. Die Verbindung ist indessen nicht nach Art jener inniger Durchdringung wie wir sie für die Rumpfnerven kennen, vielmehr kreuzt der motorische Stamm dem sensibeln und geht jenseits von der Kreuzungsstelle direct in die Kaumuskelzweige über. Nur zwei Zweige erfahren einen wirklichen Austausch der Bahnen, der mit dem N. mandibularis gehende N. mylohyoideus, und der in Begleitung der Muskelnerven gehende N. buccinatorius."

The foregoing investigations were undertaken in the case of man. Willems ('11) has stated that in the rabbit "rien n'est plus facile que d'enlever le ganglion et les branches qui en dérivent, in respectant la racine motrice avec toutes les branches motrices, à l'exception du mylohyoïdien qui se mêle intimement avec les fibres du nerf dentaire inférieure à la sortie du crâne."

The inferior maxillary division of the fifth cranial nerve innervates the masseter, temporal, external pterygoid, internal



pterygoid, tensor veli palatini, tensor tympani, mylohyoid, and anterior belly of digastric.

Though the innervation of the tensor veli palatini—both in man and in other mammals—by the fifth nerve has been described by anatomists, the evidence afforded by cases of disease and by division of the roots of the nerve in man is equivocal. Krause found no anomalies in the position of the palate after extirpation of the Gasserian ganglion and division of the motor root. Cushing found a marked asymmetry of the palate in four cases, and elicited movements of the palate by electrical stimulation of the peripheral stump of the fifth nerve in one case. Davies found asymmetry of the palate in five, and no asymmetry in twenty-one, of twenty-six cases operated on by Horsley; and he records that in three cases Horsley stimulated the peripheral end of the divided fifth nerve without any movement of the palate resulting. Davies concluded that “the balance of evidence seems to show that the fifth nerve has nothing to do with the innervation of the palatal muscles.”

Beever and Horsley ('88) stated that in *Macacus sinicus* movements of the palate occurred on intra-cranial stimulation of the vago-accessorius, but did not occur on intra-cranial stimulation of the seventh nerve. They did not state whether movements of the palate did or did not occur on intra-cranial stimulation of the fifth nerve.

Davies further states that “no change has been observed to follow excision of the Gasserian ganglion, either in the tenseness of the drum or the increased power of the individual, when tested with a Galton whistle, to appreciate high-pitched sound,” and consequently discards the innervation of the tensor tympani by the fifth nerve.

To obtain additional information on these matters Sir Victor Horsley was good enough, at my request, to divide the roots of the fifth cranial nerve proximal to the Gasserian ganglion in two monkeys (*Macacus cynomolgus*). The wounds healed by first intention. The animals were allowed to live for thirty days, and then killed by an overdose of

chloroform. The muscles and nerves, both on the cut and uncut (normal) side, were then dissected out. Sections of the muscles were stained by van Giesen's method. The nerves were stained by osmic acid and examined in transverse section.

All the masticatory muscles, including the tensor veli palatini and tensor tympani, together with the mylohyoid and anterior belly of the digastric, showed evidence of degeneration—loss of transverse striation, increase in the number of nuclei, and proliferation of the interstitial tissue.

The tensor veli palatini and tensor tympani are, consequently, innervated by the fifth cranial nerve, and belong to the group of masticatory muscles. This conclusion agrees with that obtained by investigation of their development. The Anlage of the masticatory muscles divides into an internal lamina, giving rise to the internal pterygoid, pterygo-tympanicus or tensor veli palatini, and tensor tympani; and an external lamina, giving rise to the external pterygoid, temporal and masseter.

It may be added that no degenerative changes were found in the levator veli palatini—a muscle which is developed from the pharyngeal musculature, and is innervated by a branch of the pharyngeal plexus (Cords) from the vaso-accessorius (Beever and Horsley).

In the muscle-nerves on the uncut (normal) side medullated fibres of all sizes were present from a diameter of under  $4\mu$  up to a certain maximum. This maximum was  $12.8\mu$  in the nerves passing to the tensor veli palatini, internal pterygoid, external pterygoid, temporal, masseter, and anterior belly of digastric;  $11.4\mu$  in the nerve to the mylohyoid, and  $5.6\mu$  in the nerve to the tensor tympani.

Medullated nerve-fibres were also present in the muscle nerves on the cut side, of all diameters from one under  $4\mu$  up to the same maxima as in the corresponding nerves on the uncut side, e.g. it was  $12.8\mu$  in the branch of the mylohyoid nerve to the anterior belly of the digastric and  $11.4\mu$  in the branch to the mylohyoid muscle. The number of medullated nerve-fibres in the branches on the cut side formed from 35 to

39 per cent. of the number found in the corresponding nerves on the uncut side. This percentage held though the absolute numbers were different in the two animals. Thus in animal "A" the number of medullated nerve-fibres in the trunk of the mylohyoid nerve on the uncut side was 906; on the cut side it was 335, = 36 per cent. In animal "B" the number of medullated nerve-fibres in the trunk of the mylohyoid nerve on the uncut side was 736, on the cut side it was 280, = 37 per cent.

The persisting medullated nerve-fibres in the muscle branches on the cut side were distributed fairly evenly among the degenerated ones until near the muscles (fig. 2); in the nerve-filaments just outside the muscles the persisting and degenerated nerve-fibres were largely, though not wholly, segregated from one another (fig. 3), and the former tended to lie on one side of the filament.

To ascertain the source of these non-degenerating nerve-fibres serial sections were made through the mandibular division of the fifth nerve, from the site of operation to the point where the various branches had begun to diverge from one another. In neither animal are any medullated fibres visible in the motor root above the level of the Gasserian ganglion—all had undergone degeneration. At the level of the Gasserian ganglion, and for a little distance below, medullated fibres can be seen passing from the sensory into the motor root. They lie, for the most part, in the lateral part of the motor root, and are more sparsely scattered in its median part (fig. 4).

The ramus lateralis—which innervates the external pterygoid, temporal and masseter muscles—is formed from the lateral part of the motor root (figs. 5, 6, 7, 8); it also receives, from the ramus posterior, those fibres which form its (sensory) buccal nerve constituent (fig. 6). The ganglionated afferent fibres for the muscle branches of the ramus lateralis thus have a simple direct path.

The paths of the (degenerated) motor and ganglionated afferent fibres of the ramus medialis—which innervates the

internal pterygoid, tensor palati and tensor tympani—and of the mylohyoid nerve are more complicated. In each case the persisting afferent nerve-fibres in the motor root, accompanied by (degenerated) motor fibres, pass into those branches by two routes. The ramus medialis (figs. 5, 6, 7) is formed partly by fibres which pass downwards and inwards, from the motor root, into the ramus, partly by fibres which leave the lateral part of the motor root (fig. 6) and sweep round the back of the ramus posterior from without inwards and so enter the ramus. The relative numbers of the (degenerated) motor fibres following these two paths—internal and external—could not be determined, but it was possible to do so in the case of the persisting afferent fibres. In animal "A" the internal path contains 50 medullated fibres, whilst the ramus medialis, when fully formed, contains 219, i. e. about one quarter followed the internal path and three quarters the external one, round the ramus posterior. The ramus medialis passes through the otic ganglion, giving off, just as it enters, the branch for the tensor tympani (fig. 7), and subsequently dividing into branches for the internal pterygoid and tensor palati. The branch to the tensor tympani receives a fine filament from the otic ganglion containing (in animal "A") eight medullated fibres; above that point it contains twenty-eight medullated fibres. The branch to the internal pterygoid and tensor palati receives three fine filaments from the otic ganglion containing twenty-nine medullated fibres. The medullated fibres entering these branches from the otic ganglion are all small—under  $4\mu$  in diameter.

The mylohyoid nerve is formed partly from internal fibres (degenerated and intact) which pass from the inner part of the motor root (fig. 5), a little higher up than the direct fibres of the ramus medialis, round the back of the ramus posterior from within outwards, and thus come to lie between the ramus lateralis and the ramus posterior (figs. 6 and 7); they are joined by external fibres (degenerated and intact) from the deeper, more posterior part of the ramus lateralis, and pass inwards on the anterior aspect of the ramus posterior

(fig. 8) to take up a position on its antero-median side. Here they are joined by a filament from the otic ganglion, containing a few small medullated fibres. It was not found possible to estimate the relative numbers of intact fibres following the two paths.

It follows, from these observations, that in *Macacus cynomolgus* all the muscles which are innervated by the fifth cranial nerve receive not only direct medullated nerve-fibres from the motor root, but also afferent nerve-fibres which originate in the Gasserian ganglion. These ganglionated afferent nerve-fibres form about one third of the total number of the medullated nerve-fibres passing to each muscle. They are of all sizes up to the same maximum diameters as are found in the corresponding intact branches of the opposite side. The ramus medialis and mylohyoid branches also receive a few fine medullated fibres from the otic ganglion.

The proportion of ganglionated afferent nerve-fibres found in the muscle-branches of the trigeminus is thus closely similar to that shown by Sherrington to exist in the branches of spinal nerves passing to skeletal muscles.

Examination, by serial sections, of the mandibular division of the fifth nerve in man (figs. 9-20) showed similar results. The motor root receives fibres, just below the Gasserian ganglion, from the ramus posterior. The fibres of the ramus lateralis pass directly from the motor root into the ramus. The ramus medialis and the mylohyoid nerve are formed from fibres which leave the motor root and pass, some inside, some outside the ramus posterior, and then join to form these two branches. In some respects the constitution of the ramus medialis and of the mylohyoid nerve is even clearer than is the case in *Macacus*, owing to the inner and outer fibres of the mylohyoid nerve being situated distinctly more proximal—nearer the Gasserian ganglion—than are the inner and outer fibres of the ramus medialis, and also owing to the separation of the fibres of the ramus posterior into groups.

Examination by serial sections of the mandibular division

of the fifth nerve in the rabbit and dog gave the same results in regard to the entry of sensory fibres into the motor root, and the constitution of the ramus lateralis, ramus medialis, and mylohyoid nerve.

These observations show that in *Macacus*, man, rabbit and dog, the muscles innervated by the fifth cranial nerve receive afferent fibres, which originate in the Gasserian ganglion, and pass into the motor root. The motor and ganglionated afferent nerve-fibres of the ramus lateralis have a simple direct path; those of the ramus medialis and of the mylohyoid nerve have, for a space, a double course, being divided by the ramus posterior into two groups which again unite to form those nerves (fig. 21). The reasons for this curious path are doubtful—the phenomena suggest a downward growth of the ramus posterior occurring later than that of the muscular branches, and splitting up those destined for the ramus medialis and mylohyoid nerve. This actually occurs in rabbit embryos—the mylohyoid nerve is present in the  $5\frac{1}{4}$  mm. stage, the rami medialis and lateralis are developed in the 8 mm. stage, the ramus posterior not until the 9 mm. stage. I could not obtain embryos of *Macacus*, man or dog.

Information as to the end-organs of the afferent nerve-fibres of the masticatory muscles is as yet scanty and incomplete. Cipollone has stated that muscle-spindles are present in the masseter and pterygoid muscles.

**Mesencephalic Root of the Fifth Nerve.**—May and Horsley ('10) showed that practically all the axons of the globular cells of the mesencephalic root of the fifth nerve leave the pons by the motor root of that nerve, that destruction of it does not cause either motor or sensory loss, that stimulation of the root on the cut surface of the mesencephalon produces no effect on the muscles of mastication unless the excitation spreads to the pontine masticatory nucleus, and "that avulsion of the peripheral branches of the inferior division causes chromatolysis in the mesencephalic root cells, a result suggesting that these axons run in the peripheral



branches, though examination by the Marchi method has failed to reveal them."

Willems ('11) also found chromolytic changes in the mesencephalic nucleus after avulsion of the individual motor branches of the fifth.

Though the observations described in this paper show the existence of ganglionated afferent nerve-fibres in the muscle-branches of the fifth nerve, they leave untouched the difficult question of the peripheral distribution and function of the axons of the mesencephalic root.

I owe many thanks to Sir Victor Horsley for performing the operations described above. The expenses have been defrayed by a grant from the Bristol University Colston Committee.

September 18th, 1912.

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### EXPLANATION OF PLATES 33-36.

Illustrating Dr. F. H. Edgeworth's paper “On the Afferent Ganglionated Nerve-fibres of the Muscles Innervated by the Fifth Cranial Nerve; and on the Innervation of the Tensor Veli Palatini and Tensor Tympani.”

#### LIST OF ABBREVIATIONS.

*br<sup>a</sup>, r. med.* Branches of ramus medialis. *buc. n.* Buccal nerve. *ext. aff. r. med.* External afferent fibres of ramus medialis. *ext. f<sup>a</sup>. mylohy. n.* External fibres of mylohyoid nerve. *ext. f<sup>a</sup>. r. med. & mylohy. n.* External fibres of ramus medialis and mylohyoid nerve. *int. aff. f<sup>a</sup>. mylohy. n.* Internal afferent fibres of mylohyoid nerve. *int. f<sup>a</sup>. mylohy. n.* Internal fibres of mylohyoid nerve. *int. f<sup>a</sup>. r. med.* Internal fibres of ramus medialis. *mot. r.* Motor root. *mylohy. n.* Mylohyoid nerve. *n. int. pty. & t. pal.* Nerve to internal pterygoid and tensor palati. *n. t. tymp.* Nerve to tensor tympani. *n. temp. ext. pty. & ma.* Nerve to temporal, external pterygoid and masseter. *ot. g.* Otic ganglion. *r. lat.* Ramus lateralis. *r. med.* Ramus medialis. *r. post.* Ramus posterior. *sens. f<sup>a</sup>.* Sensory fibres entering motor root.

Note.—In fig. 14 the directing line from “*int. f<sup>a</sup>. mylohy. n.*” should pass directly upwards to the group of fibres on the periphery of the nerve. cf. fig. 15.

[Figs. 1-8 are from *Macacus*.]

Fig. 1.—Right masseter nerve: roots of left fifth cranial nerve divided thirty days previously.

Fig. 2.—Left masseter nerve: roots of left fifth cranial nerve divided thirty days previously.

Fig. 3.—Left anterior digastric nerve, close to muscle: roots of fifth cranial nerve divided thirty days previously.

Figs. 4-8.—Sections from a serial series made through the fifth cranial nerve, the roots of which had been divided thirty days previously. Fig. 4 is the most proximal.

Figs. 9-20.—Sections from a serial series made through the fifth cranial nerve of man. Fig. 9 is the most proximal.

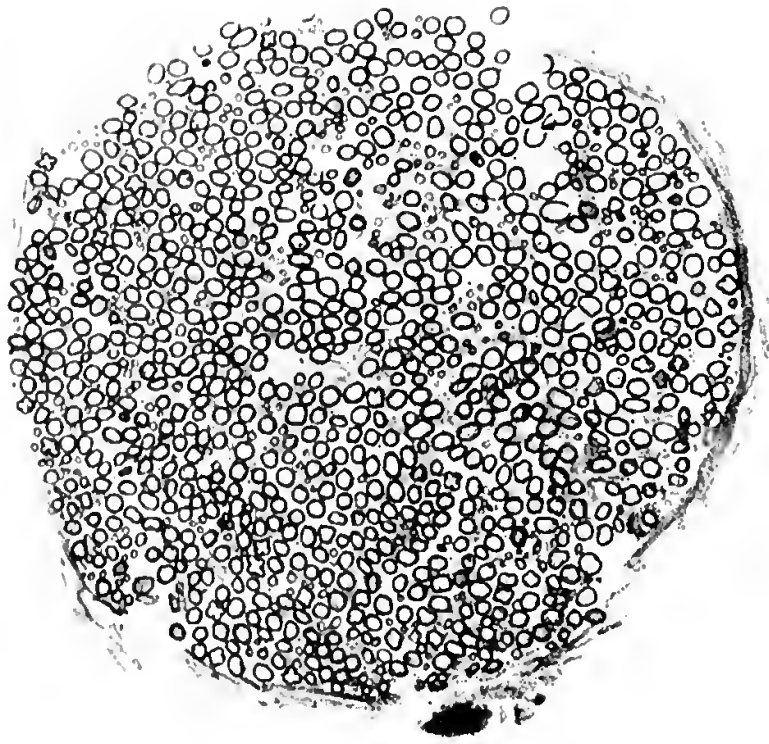
Fig. 21.—Diagram representing the paths of the motor and afferent constituents of the muscular branches of the fifth cranial nerve. Motor fibres are represented by a thick dotted line, afferent fibres by a thin dotted line. To show these paths on one plane, the constituents of the mylohyoid nerve are depicted lying internal to those of the ramus medialis; in reality they are more proximal.



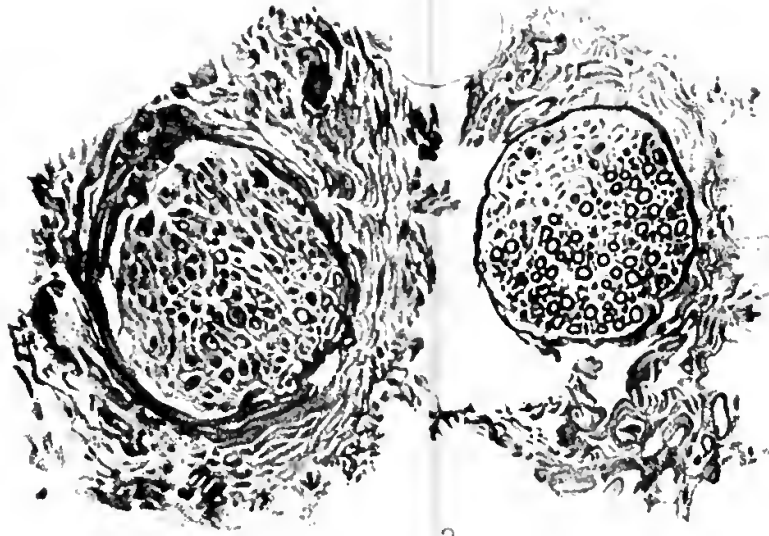




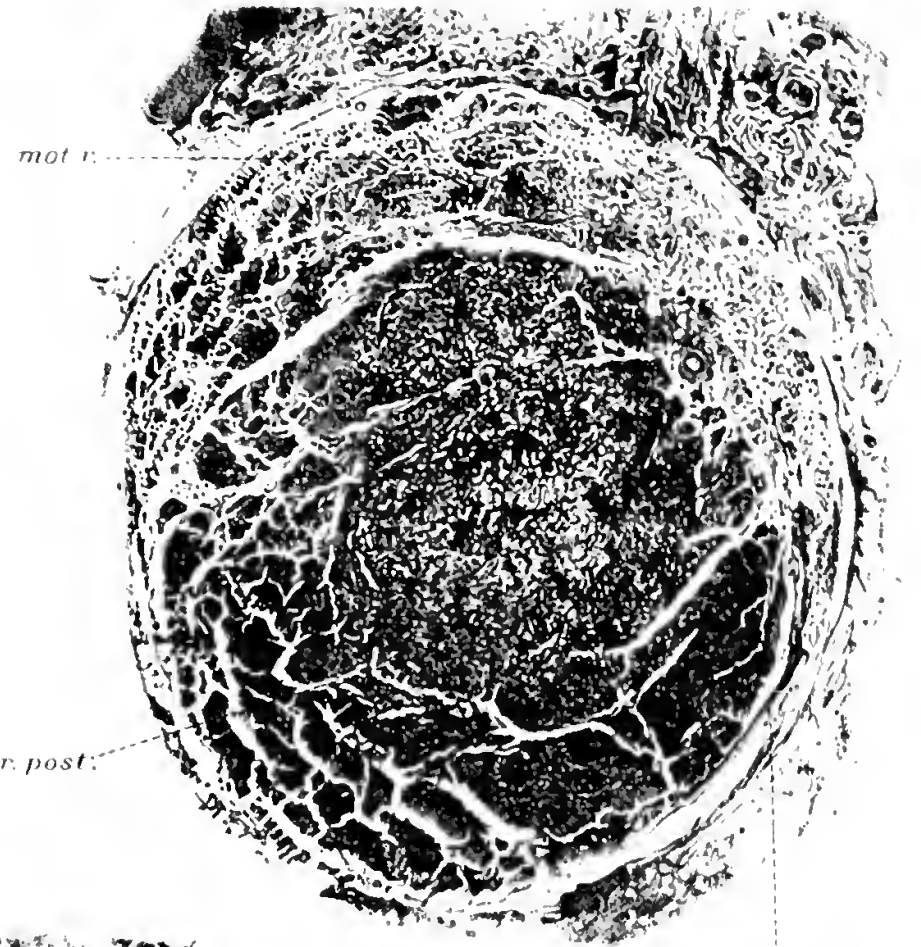




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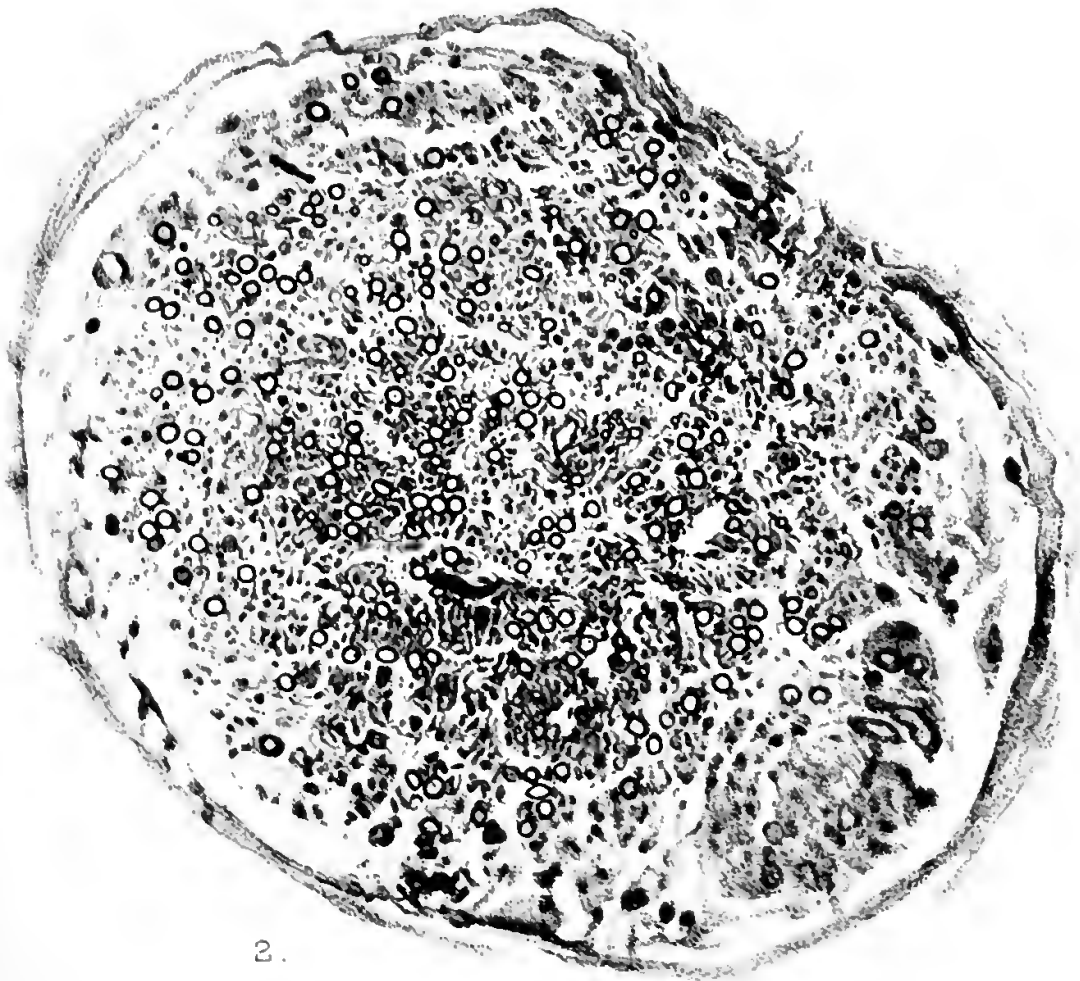


*mot. r.*

*r. post.*

5.

*int. all. f. s. mylohy. n.*



2.



*mot. r.*

*r. post.*

4.



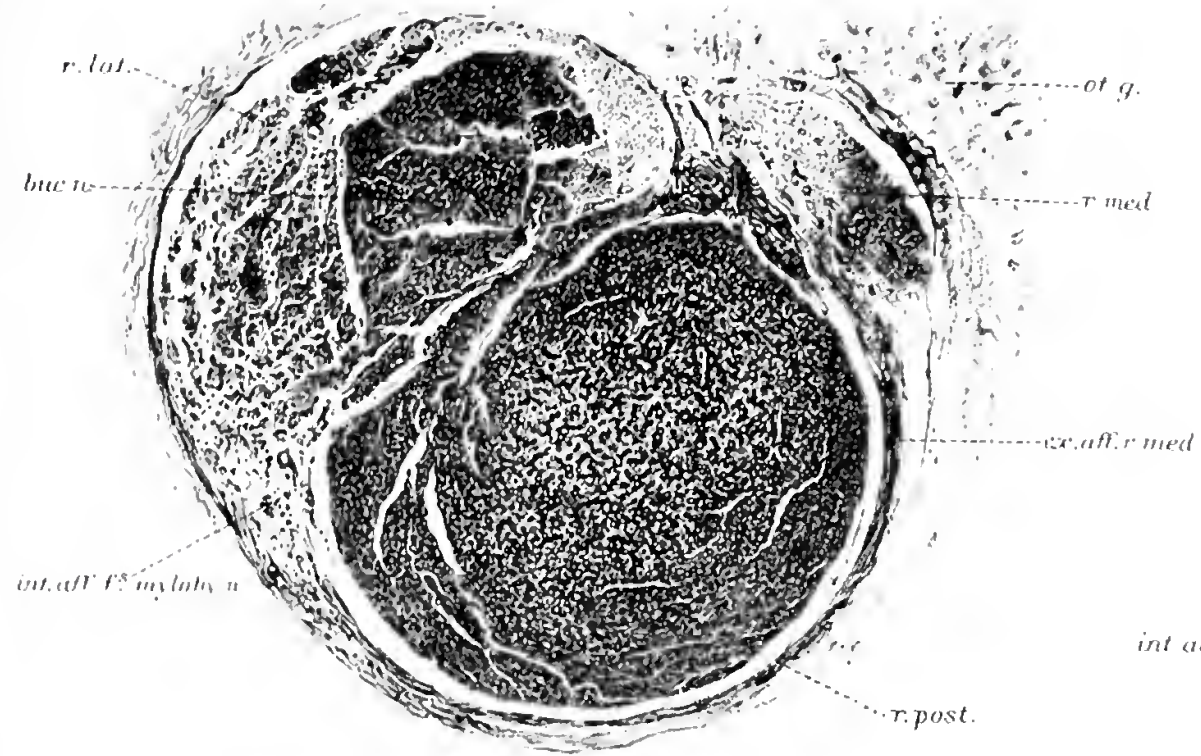




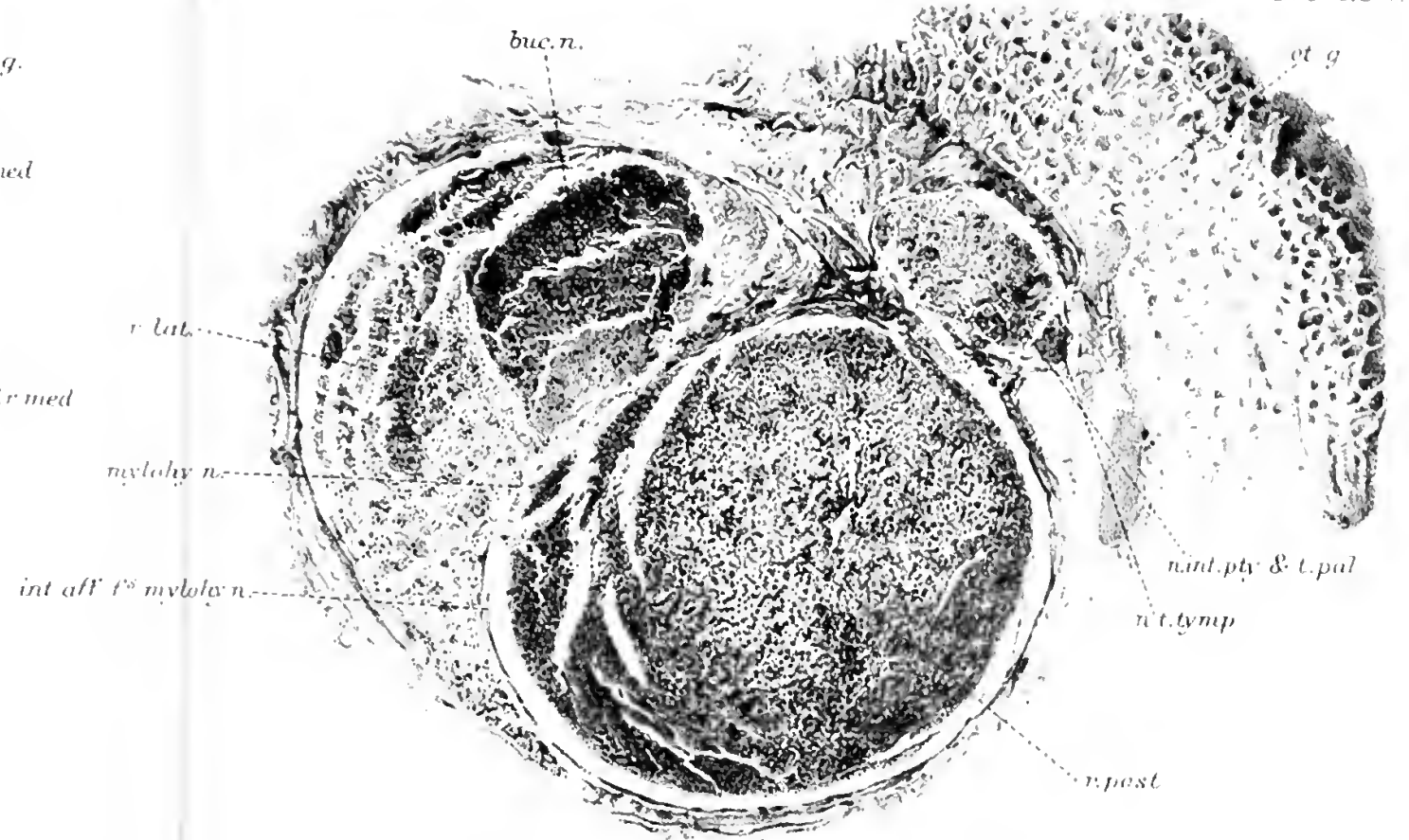








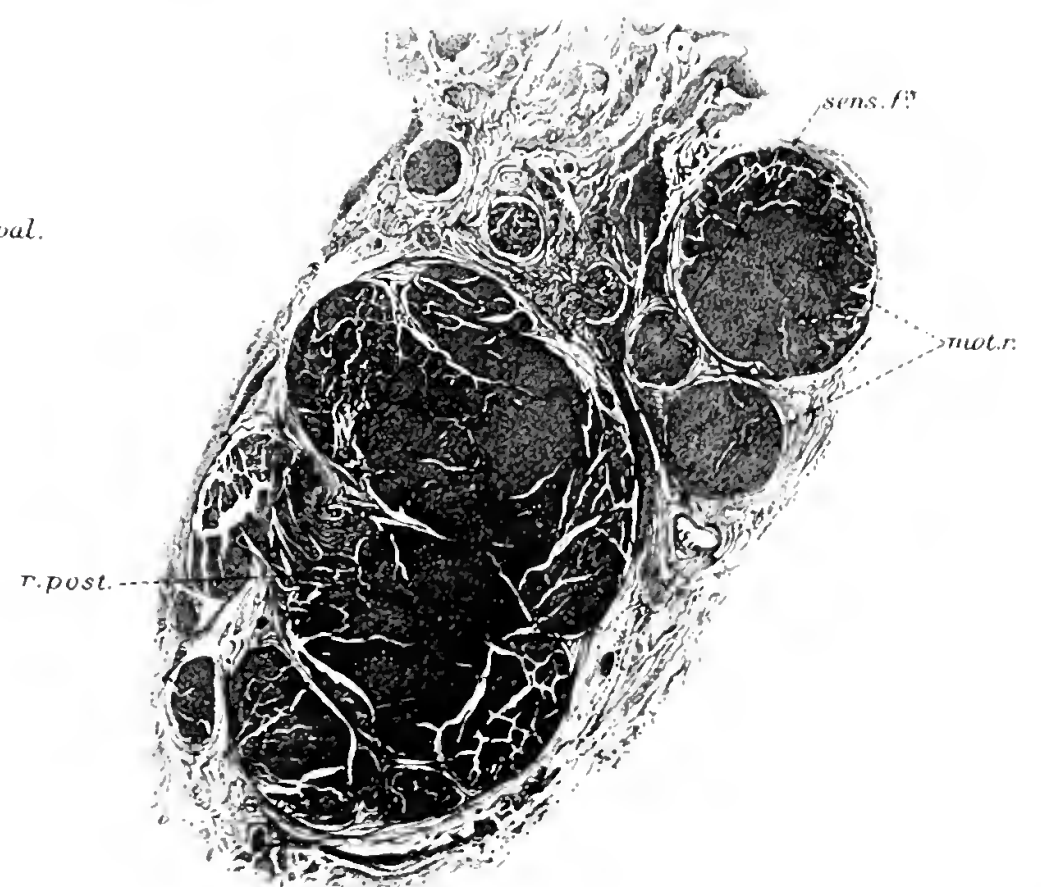
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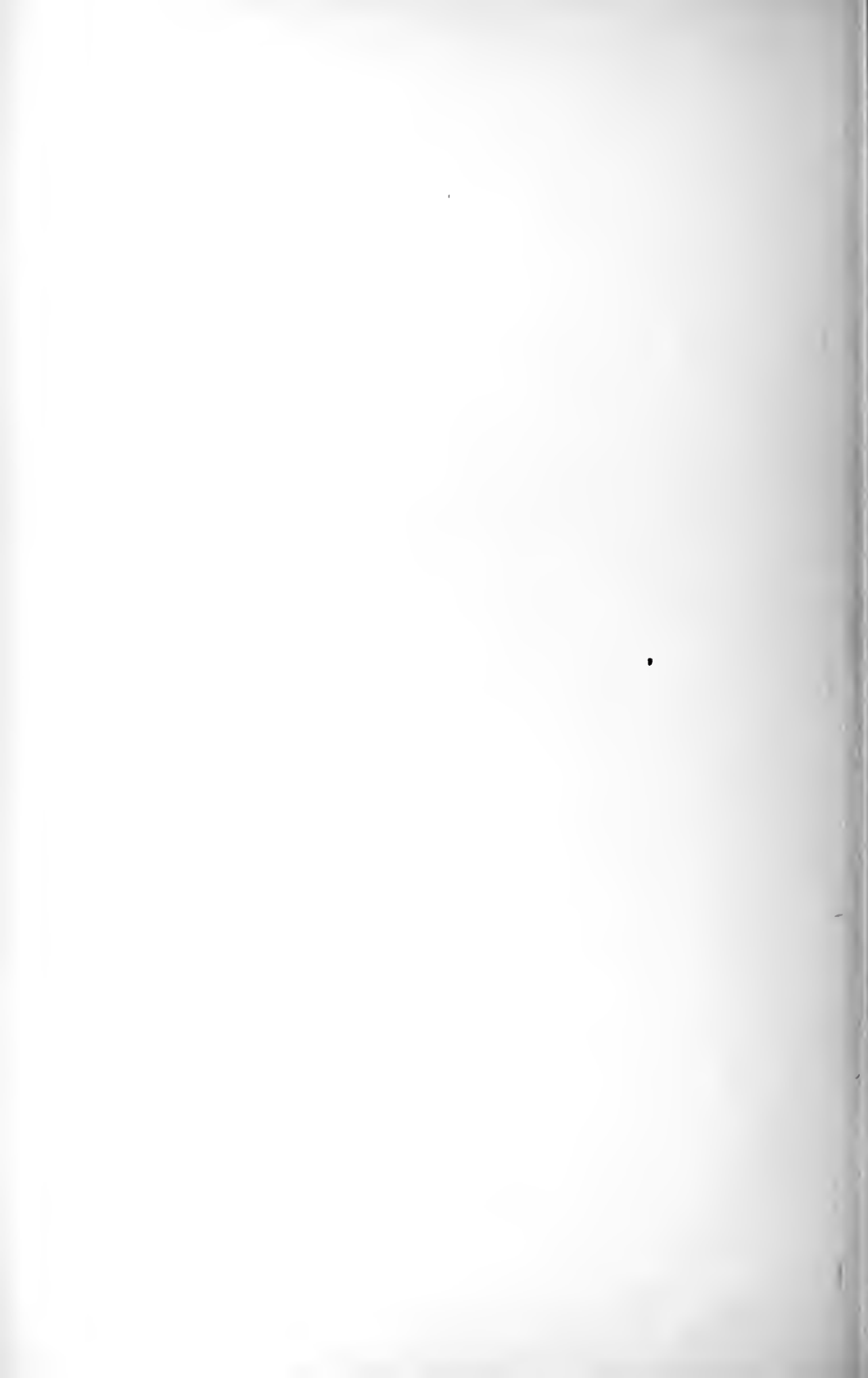
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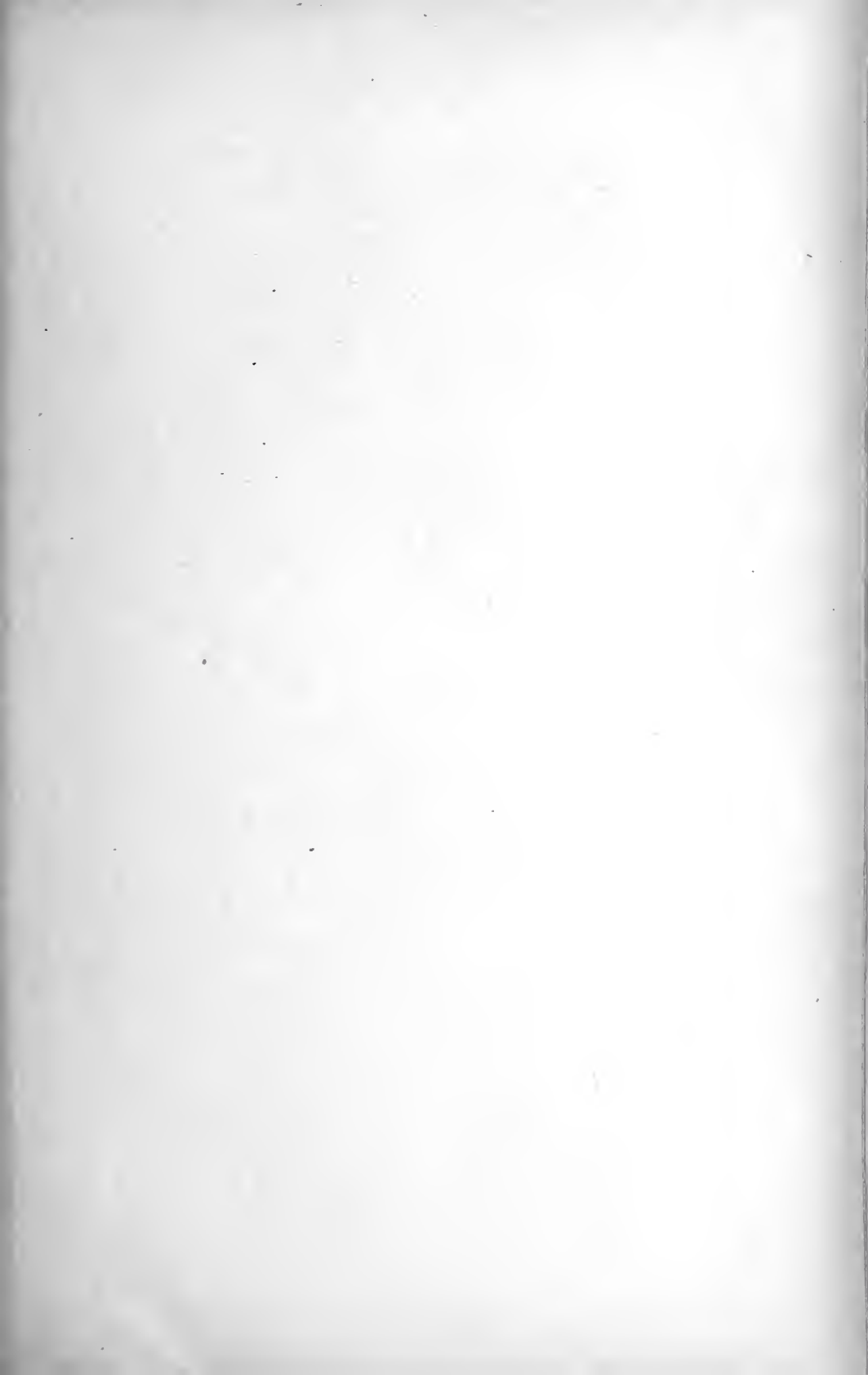


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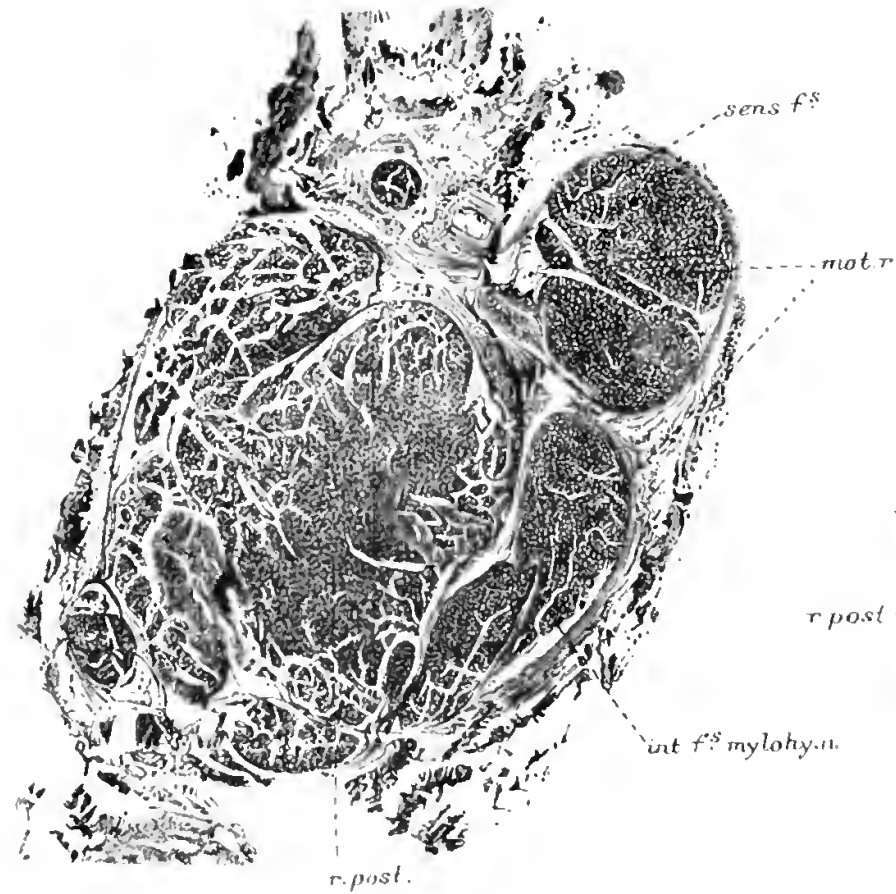




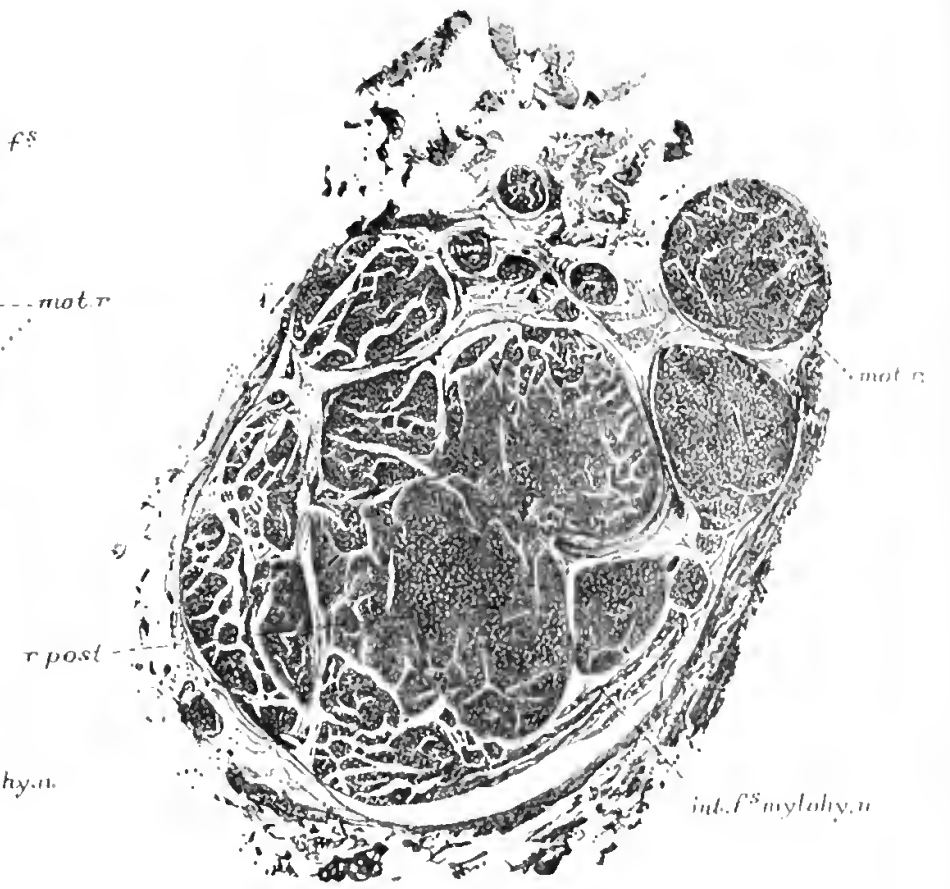




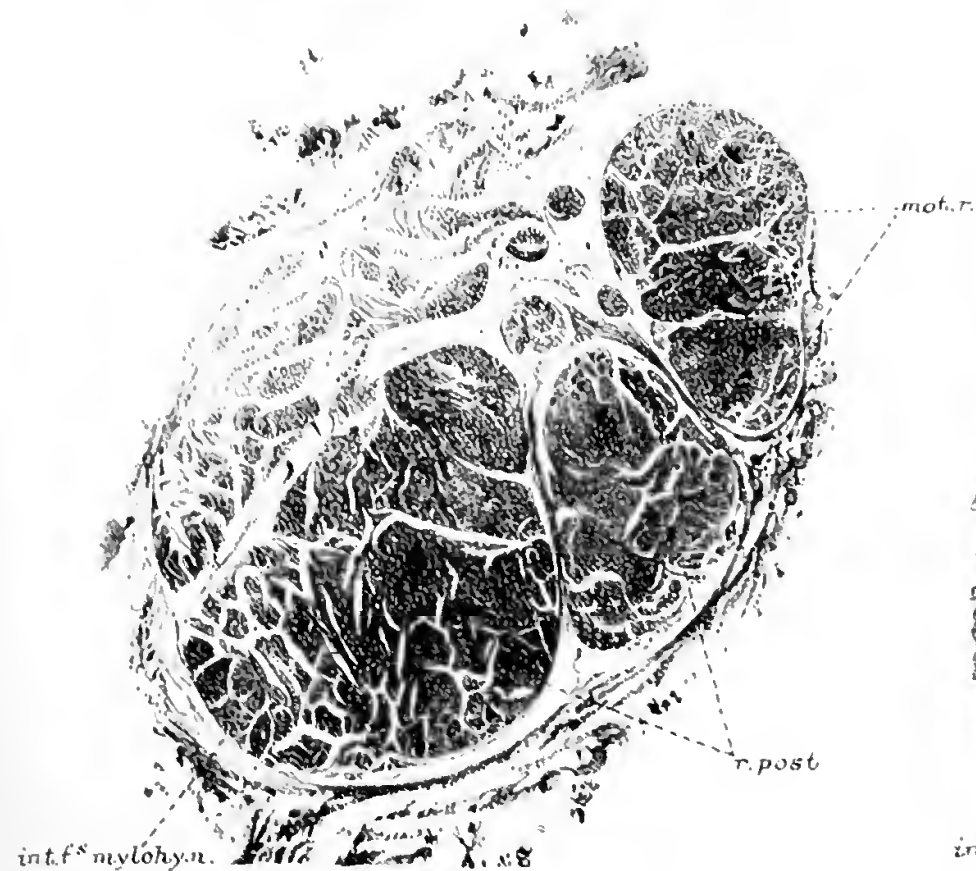
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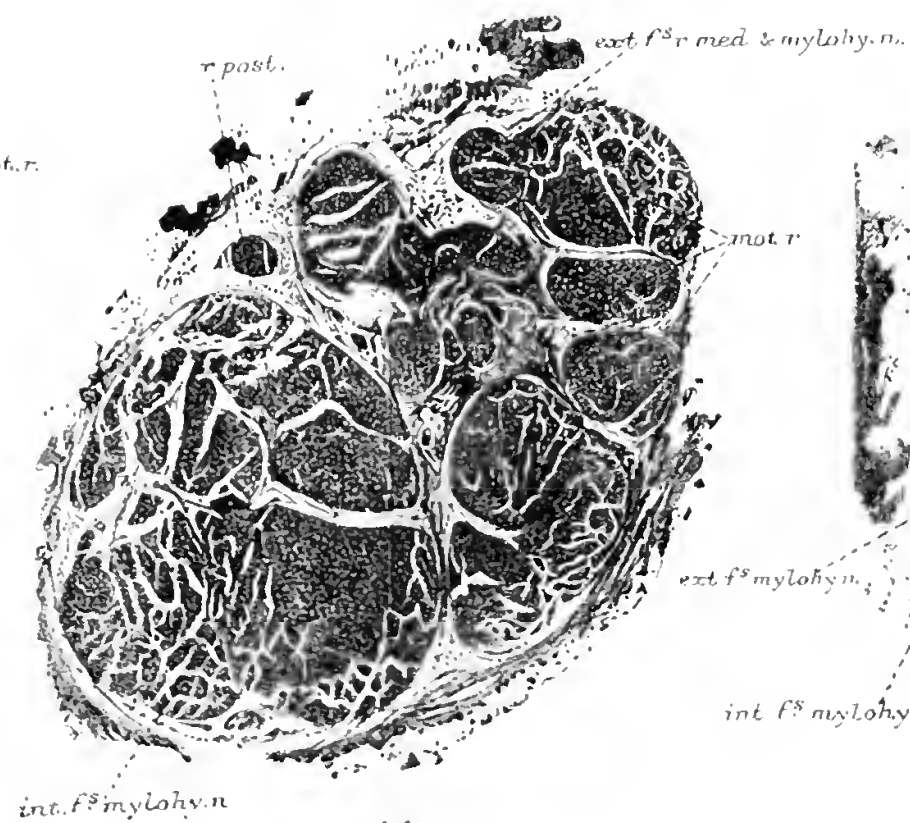
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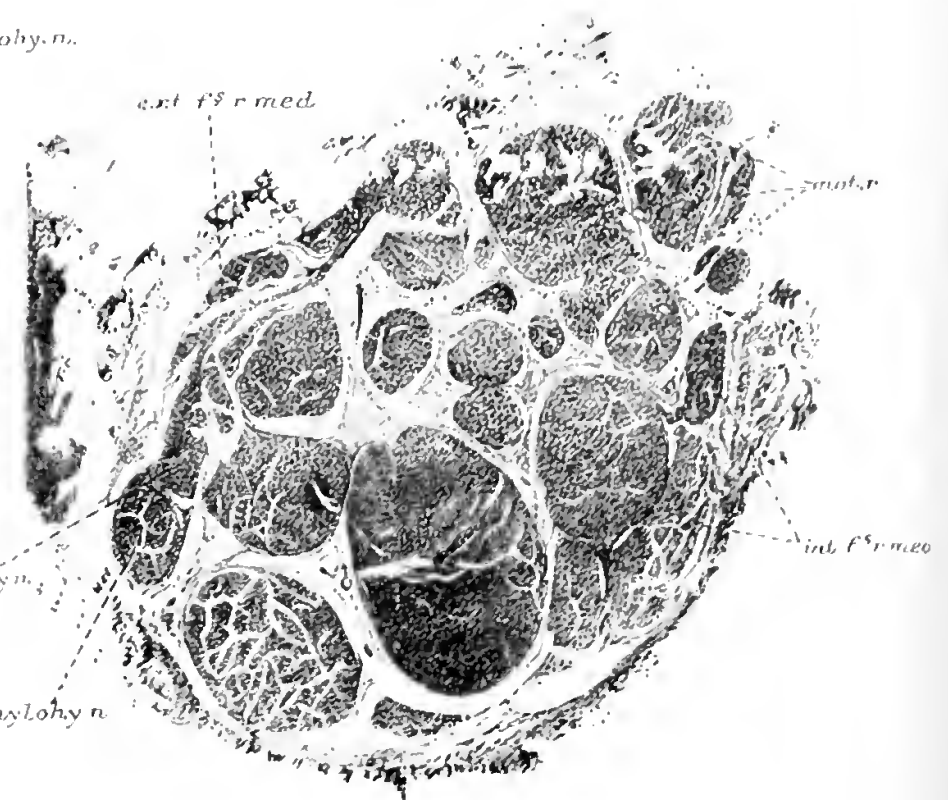
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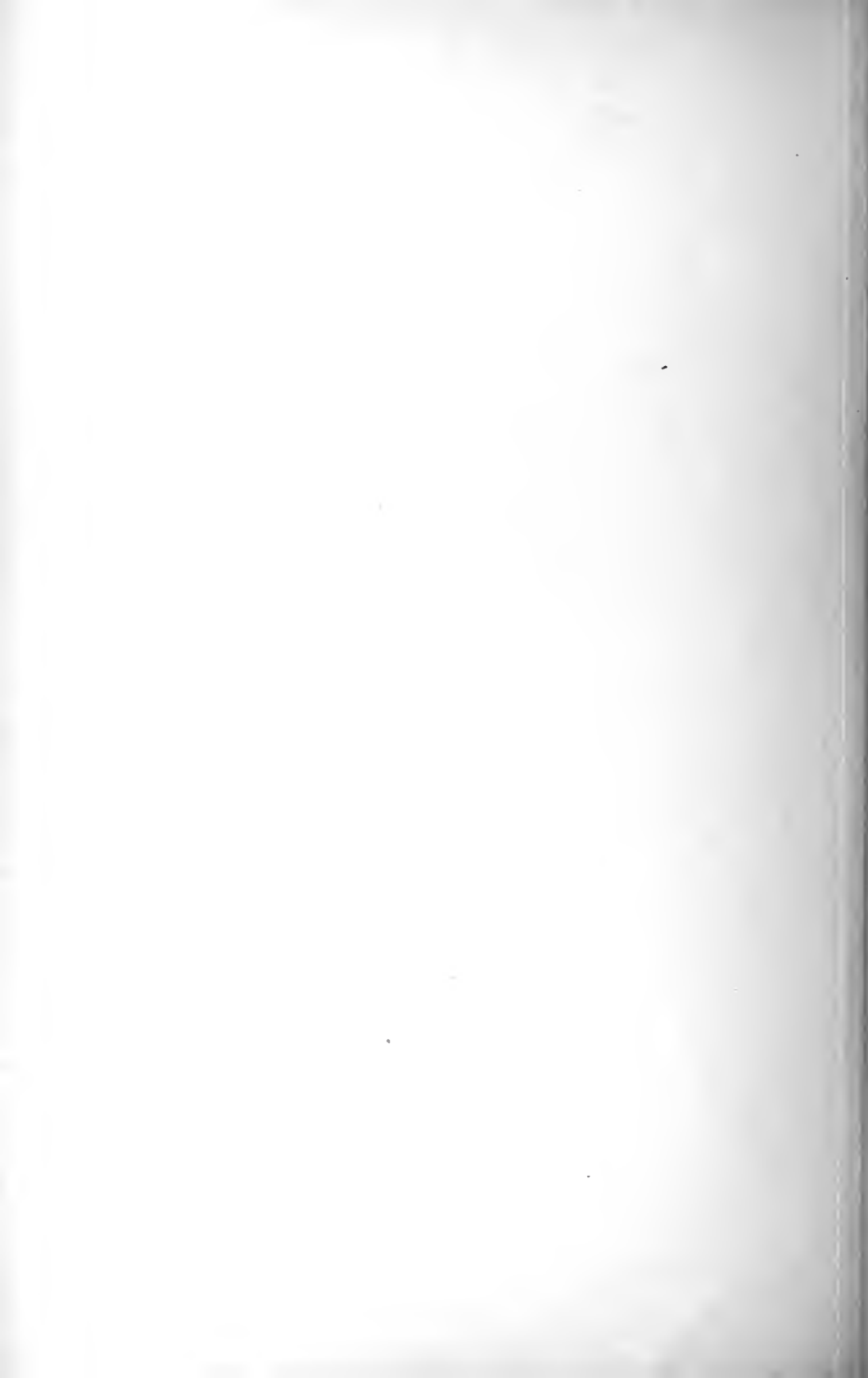


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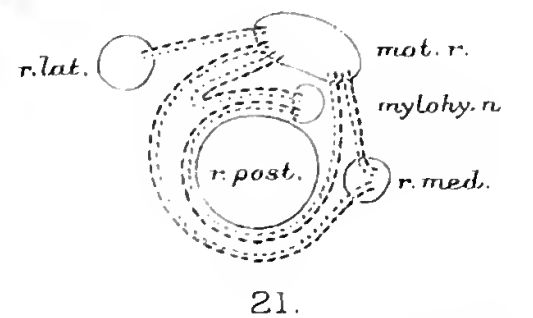
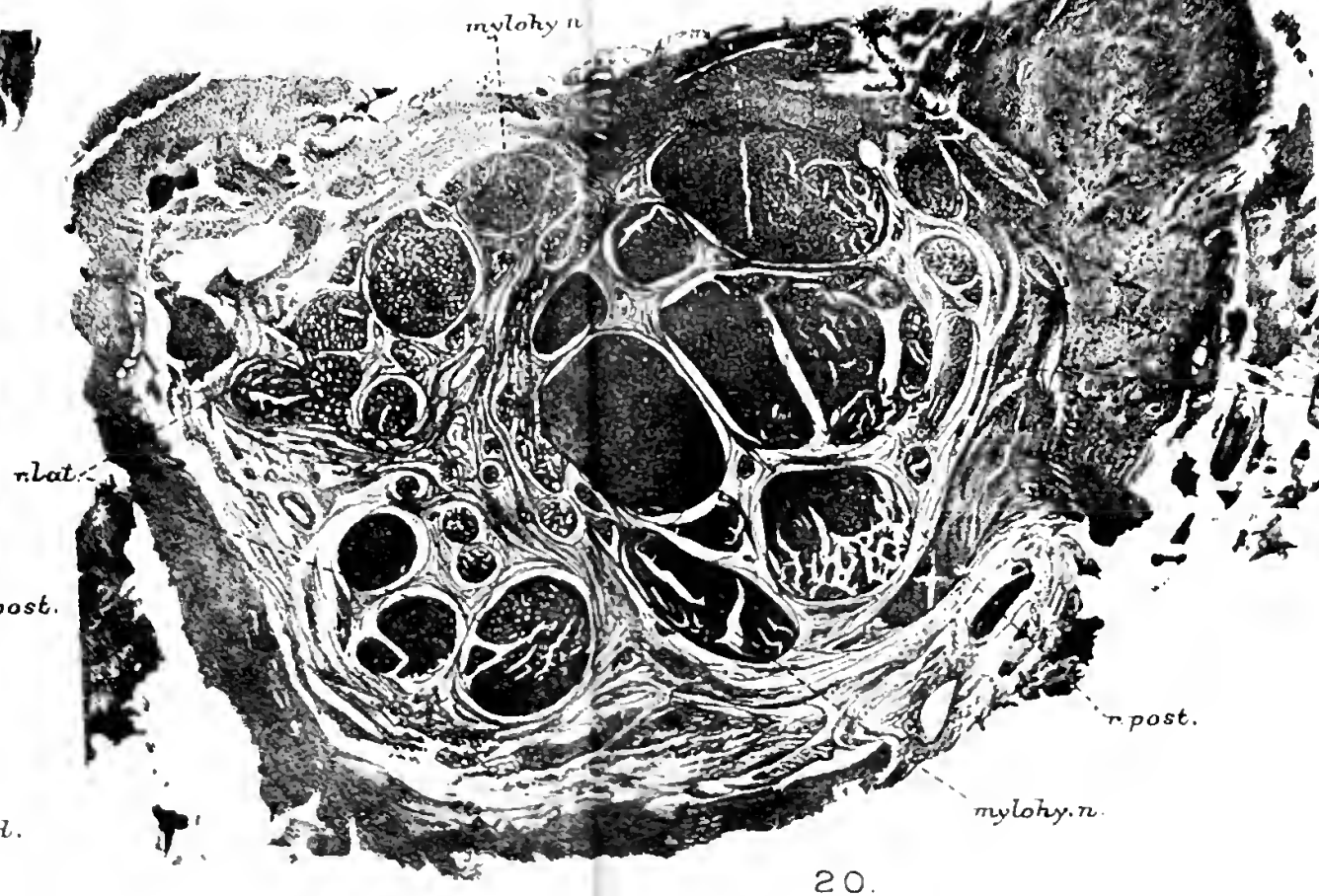
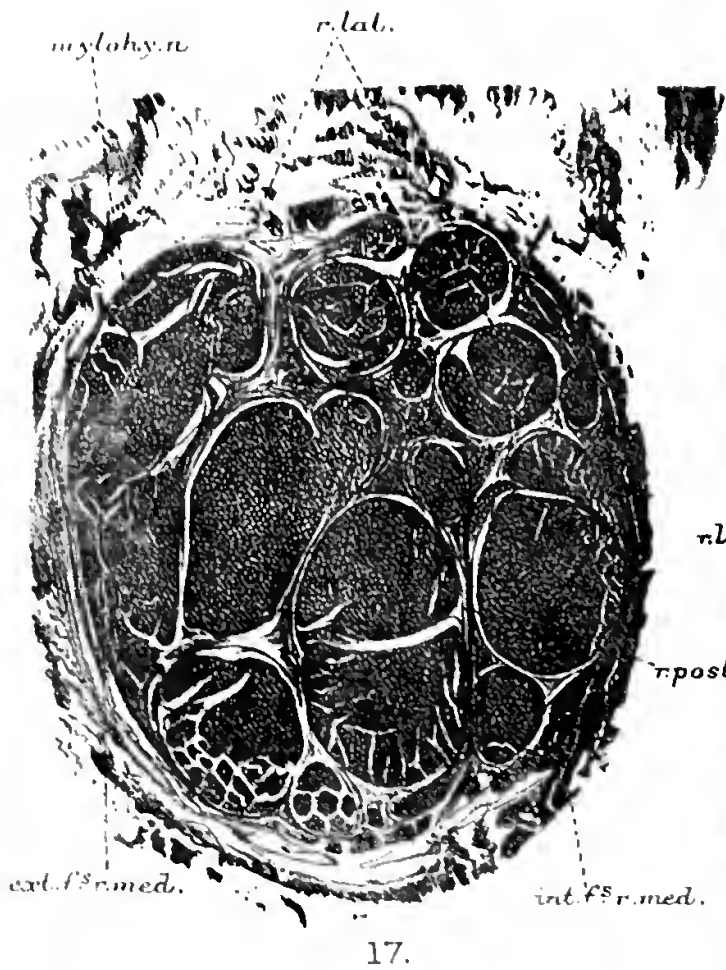
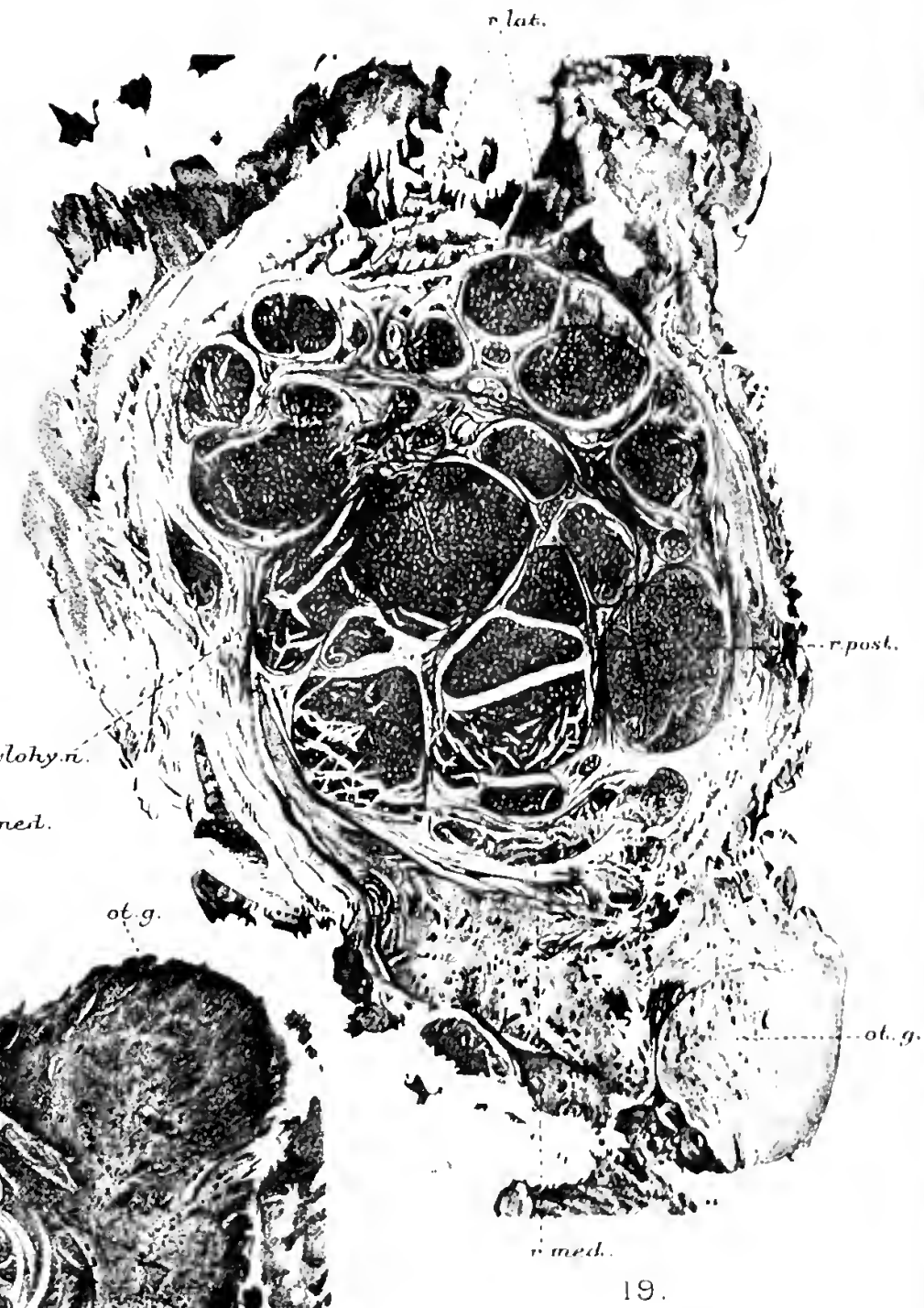
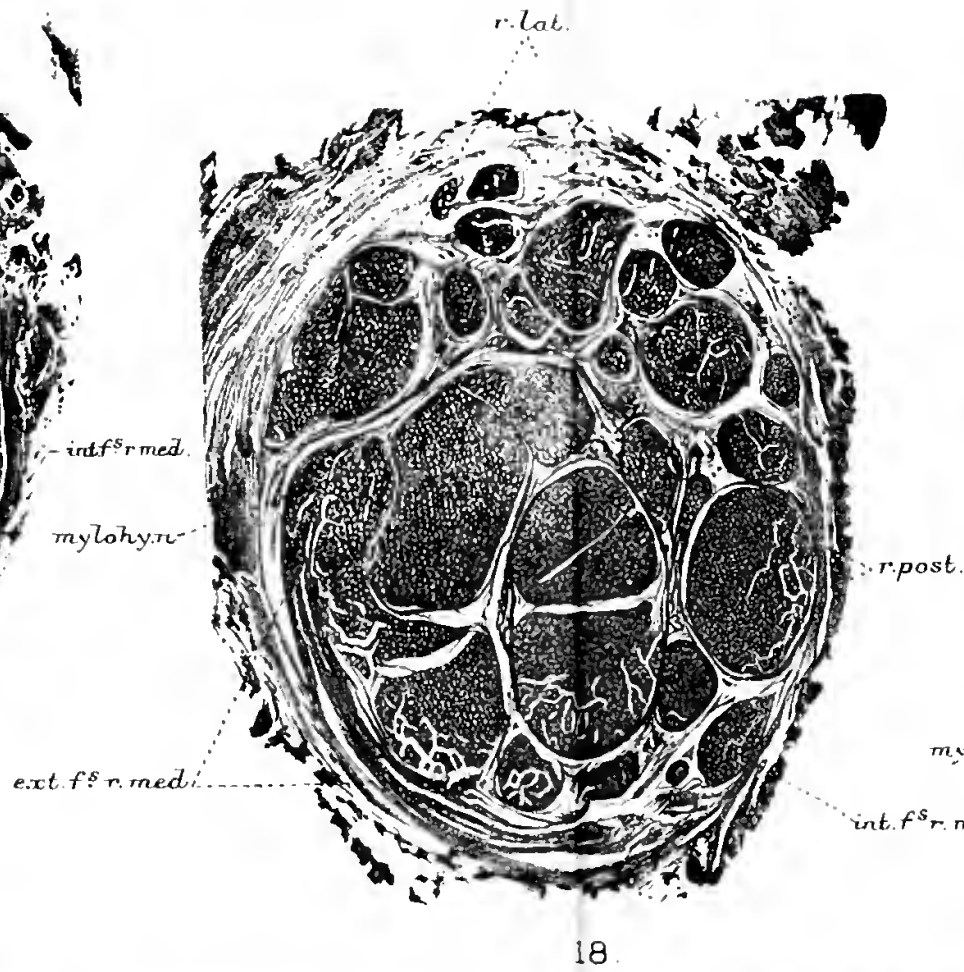
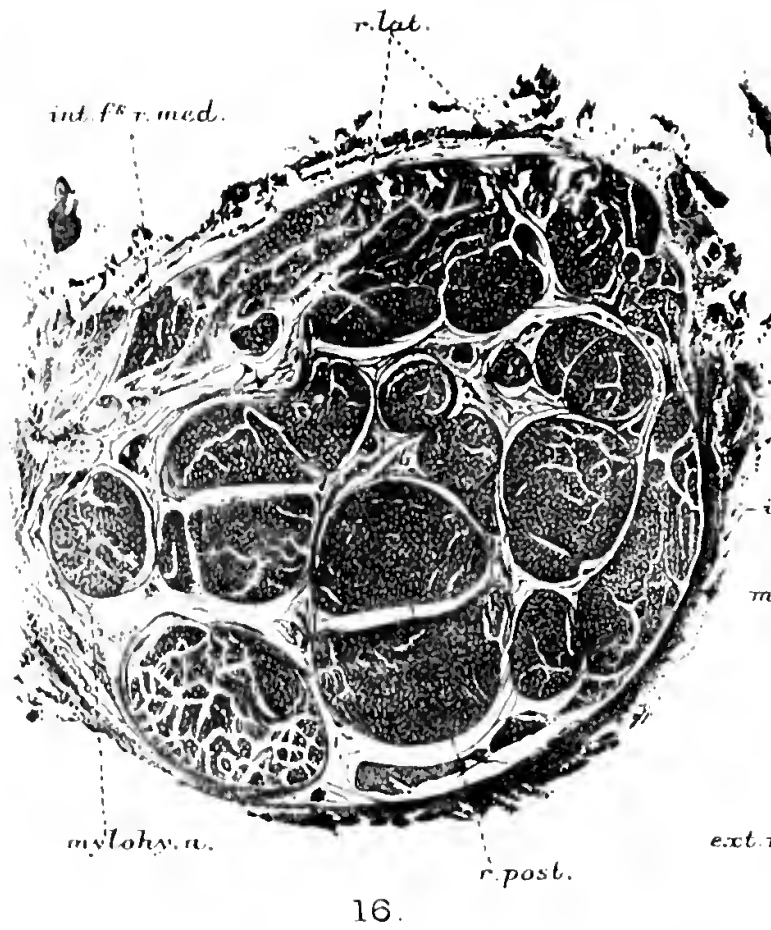




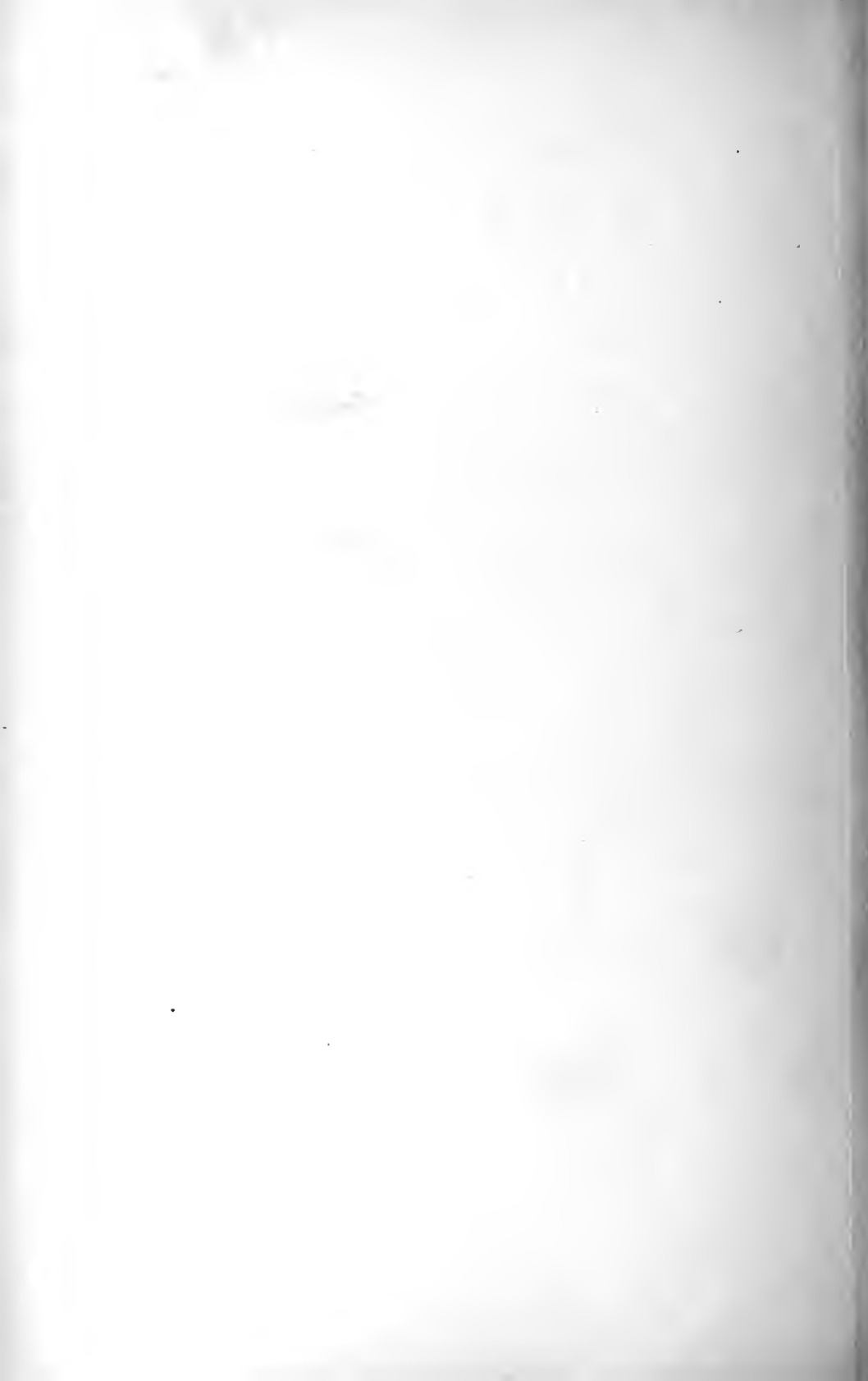












# On the Nematodes of the Common Earthworm.

By

**Gilbert E. Johnson, M.Sc.(Birm.),**

University of Birmingham; Board of Agriculture and Fisheries  
Research Scholar.

(Studies from the Research Department in Agricultural Zoology,  
University of Birmingham.)

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With Plate 37 and 2 Text-figures.

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## INTRODUCTION.

### (1) Survey of the Literature.

It has long been a matter of common knowledge that larval nematodes inhabit the common earthworm, *Lumbricus terrestris*, Linn. They are found both in the cœlom and in the nephridia. Those occurring in the cœlom

are enclosed in cysts or capsules, by which movement is restricted or entirely prevented. Those inhabiting the nephridia, on the other hand, are free and active. The encysted, cœlomic form has long been known as *Rhabditis pellio*, having been named by Schneider (1) as early as 1866. The nephridial form is generally believed to be the same species, but it was not mentioned by Schneider, and I have not been able to find that its identity has been determined by any subsequent investigator, as the following brief survey of the literature will show.

In 1845 Dujardin, according to Bastian (2), recorded the existence of nematodes in the general body-cavity of the earthworm. These he placed in the genus *Rhabditis*. He found that they developed in prodigious numbers, forming whitish masses in the vessels in which he had kept earthworms with moss and damp soil. Bastian says that Dujardin also described a nematode, *Dicelis filaria*, occurring in great abundance in the nephridia of the earthworm.

In 1858 Lieberkühn, according to von Linstow (3), showed that the "*Filariæ* of the earthworm," after the death of their host, creep out of the cysts, moult, and in a few days develop into mature worms.

In 1864 and 1865 Lankester (4, 5) mentioned the nematodes as occurring in the lobes of the seminal vesicles, in the posterior end of the cœlom and imbedded in the muscular layer of the body-wall.

Hitherto only the larvæ had been investigated, but in 1866 Anton Schneider (1) described the adults. He stated that they occur "in damp earth and putrefying substances." These terms he must have used to signify dead earthworms decaying in moist earth, for he says—"The larvæ occur encysted in the body cavity of earthworms, especially on the septa," and adds that, as Lieberkühn was the first to point out, they become sexually mature on the decay of the worms. Schneider named the species *Pelodera pellio*.

In 1873 Bütschli (6) described the adult males and females obtained from decaying earthworms in greater detail and with



good figures. He noticed certain structural differences between his form and Schneider's but regarded these as unimportant. He altered Schneider's name *Pelodera pellio* to *Rhabditis pellio*, *Rhabditis* being Dujardin's name for the larvæ which he found in the earthworm.

Von Linstow (3) in 1882 described the larvæ as well as the adult males and females, and, using a decaying worm, actually developed the encysted larvæ into the sexually mature *Rh. pellio*.

Railliet (7) in 1893 merely quoted Schneider's description.

Keng (8) in 1895 watched the encystment of the cœlomic form, but he does not show that he was aware of the identity of the nematodes with which he was dealing.

In 1897 von Erlanger (9), working on the segmentation of the egg, made a pure culture of *Rh. pellio* from strips of the body-wall of an earthworm placed on soil which had been first sterilised and then moistened. He obtained large numbers in this way.

Maupas (10) in 1899 made extensive cultures of *Rh. pellio* with an artificial food-medium. He probably used for parents the encysted larvæ from the freshly killed worm or the mature adults from decaying worms, for, while he speaks about the encysted, cœlomic form, strangely enough he does not mention the active, nephridial larvæ.

In 1900 de Ribaucourt (11) described the excystation of the encysted larva. He mentions the nephridial form and explains its probable method of entry. He did not attempt to determine its identity or show that he knew that of the encysted form, but he evidently regarded them as belonging to the same species.

Shipley (12) in 1902 summarised the work of the earlier writers. Like them he says nothing of the nematode in the nephridia.

K. C. Schneider (13) in 1902 mentioned the encysted larvæ of *Rh. pellio* occurring in the cœlom. He also says that nematodes, which, according to Anton Schneider, belong to *Rh. pellio*, are frequent in the lumen of the bladder of the

nephridium. But this statement is incorrect, for, as has been seen, Anton Schneider did not mention the nephridial form.

It will be seen from the foregoing survey that, while almost every writer describes the larva of *Rh. pellio* as living encysted in the coelom, there are only three who mention the nephridial larva. Dujardin called it *Dicelis filaria*, evidently regarding it as a different species from the coelomic *Rhabditis* form. De Ribaucourt mentions both forms, and though unconcerned with their identity, writes as if he believed them to be the same species. Lastly K. C. Schneider wrongly supposes that Anton Schneider identified the nephridial form as *Rh. pellio*, whereas he did not even mention it.<sup>1</sup>

Thus although the nephridial form is generally supposed to be the same species as the coelomic form, *Rhabditis pellio*, it appears that in reality its identity has never been determined.

## (2) Nature of the Research.

The present research, then, was undertaken with the object of identifying, and following out, if possible, the life-history of the active larval nematode inhabiting the nephridia, and in the hope of being able, in so doing, to throw some light upon the relations of sex in the group. The work has proved exceedingly difficult, and the conclusions reached are in some cases largely hypothetical. But the subject is one of great interest, and further work should yield valuable results.

The work has been carried out in the Zoological Laboratory of the University of Birmingham, under the supervision of Professor F. W. Gamble, F.R.S., to whom I am very grateful for his unfailing assistance, by suggestion and by criticism, throughout the course of the investigation. I am deeply indebted to the Board of Agriculture and Fisheries for the award of a research scholarship in Agricultural Zoology, with the aid of which the latter part of the research has been

<sup>1</sup> For a reference to Örley's work, see footnote, p. 622.

carried on. I have also been assisted by a grant which was made me at the commencement of the work from the Endowment of Research Fund of the Birmingham Natural History and Philosophical Society.

#### OCCURRENCE.

##### (1) The Encysted Larva inhabiting the Cœlom.

The encysted form is most plentiful in the posterior end of the cœlom. In dissecting *Lumb. terrestris* a number of flattened and roughly oval bodies of brown matter varying in length from 1 to 5 mm. are to be found lying round the intestine close to the anus, in the compartments formed by the septa. These brown bodies, when placed in water and examined under the microscope, are at first too solid and opaque to show of what they consist. But in some instances a few nematodes are to be seen partly imbedded in them and partly free, their free ends waving about in the water. Occasionally large, white, rounded bodies are present, projecting from the surface. These are cysts of *Monocystis*.

When one of the brown bodies is placed in water a number of unencysted larval nematodes make their escape from it before very long. In the course of one or two days it gradually disintegrates. It is then seen to be constituted of numerous small, and occasional large, cysts of species of *Monocystis*, discarded setæ of the worm (sometimes still encased in the setigerous sac), encysted nematodes (of which some have already escaped from their cysts and others are in the act of doing so), and lastly a large quantity of loose brown cellular matter, which consists of broken-down, discoloured amœbocytes, and has held the whole mass firmly cemented together. Lankester (5, p. 104), de Ribaucourt (11) and K. C. Schneider (13, p. 425) have all described these inclusions of the cœlomic fluid at the posterior end of the body, and my observations agree with theirs.

The cyst enclosing the nematode fits round its body closely,

but, being slightly longer than the nematode, allows it to move a short distance backwards and forwards. It appears to be formed from the cast-off outer layer of the cuticle of the nematode, but it is covered with lymph-cells, some of which usually remain attached to it even after the disintegration of the brown body as a whole. It is seldom extended straight. The commonest shapes in which it is bent are the figures 3 and 8, and it is often coiled in a ring or a spiral (Pl. 37, fig. 5).

I have seen the cœlomic form in the act of emerging from the cyst on numerous occasions. One end of the cyst—usually the anterior end—is ruptured or pushed off as a cap, and the nematode works its way out by a prolonged series of writhings and contortions. Among the constituents of the disintegrating brown bodies, nematodes, covered with amœbocytes but with the cysts not yet formed underneath these, are frequently to be seen.

In addition to the nematodes congregated in the brown bodies at the tail, independent encysted individuals surrounded by cœlomic corpuscles are found in smaller numbers in all parts of the cœlom. Unencysted individuals also occur. The cœlomic form is sometimes found imbedded in the muscular layer of the body-wall or encysted on the septa (one of the positions given by Schneider, who does not mention the brown bodies in the tail and may not have seen them). Lankester (4, Pl. vii, fig. 12) gives a drawing of a nematode in the former position.

I have examined other species of earthworms besides *Lumb. terrestris*, the common earthworm, and have found the brown bodies in *Lumb. rubellus* Hoffmeister, *Allolobophora longa* Ude, *All. turgida* Eisen, and *Octolasion cyaneum* Savigny, and I do not doubt that they occur in other species as well. All except those in *All. turgida* contained encysted larval nematodes.

Though varying considerably in size and stoutness of build, the cœlomic form is always found in a larval, never in an adult, state in the freshly killed worm.

## (2) The Active Form living free in the Nephridia.

The nephridial form inhabits the cavity of the "bladder," the dilated muscular termination of the nephridial tube next to the nephridiopore. It occurs very constantly in worms of this particular species. Several are to be found in almost every nephridium. The number present varies from two or three to over a dozen. The nematodes are found in worms of all sizes. The largest and most healthy-looking appear to be infested quite as much as the weakly specimens. I have seen the nematodes in worms which are only 1.5 in. long and are so young as scarcely to be recognisable as *Lumb. terrestris*. The only part of the nephridium in which the nematodes occur is the bladder. On one occasion, certainly, I saw one in the "wide tube," but it had evidently strayed from the bladder and it soon went back.

The active form is sometimes met with in the seminal vesicles (5, p. 11).

I have examined other species of earthworms besides *Lumb. terrestris*, and have found the nephridial form present in *Lumb. rubellus* Hoffmeister, *Eisenia fœtida* Savigny, *Dendrobæna subrubicunda* Eisen, and *Octolasion cyaneum* Savigny. They are in some cases plentiful, as they are in *Lumb. terrestris*. But they are more often present in only very small numbers, and most frequently absent altogether. The nephridial form in *Oct. cyaneum* belongs to the same species as that in *Lumb. terrestris*. I have not identified those found in the other species of earthworms, but I believe them also to be the same.

The nephridial form is in an active condition, though it may exhibit very varying degrees of activity. It often remains coiled and motionless or makes only sluggish movements. At other times it writhes unceasingly. Like the cœlomic form it is always found in a larval, never in an adult, state in the freshly killed worm. Like the cœlomic form, too, it varies considerably in size and stoutness of build, but the

majority of individuals are smaller and slenderer. Its average length is .5 mm. (Pl. 37, fig. 4). The two forms appear identical in structure and proportions. But, in the undeveloped condition in which they exist in the worm, they do not exhibit features sufficiently distinctive to make identification possible, the different species of *Rhabditis* being very much alike in the larval stage. The sexually mature males provide the most important means of discriminating the different species, the disposition of the papillæ or rays of the bursa (Pl. 37, fig. 9) being the most useful diagnostic character.

In order, then, to determine the identity of the nephridial form it was necessary to rear it from the larval to the adult condition, and with this object, as well as with that of investigating the sexual phenomena, cultural methods were employed.

#### CULTURAL METHODS.

The researches of Maupas (10, 14), extended by those of Potts (15), on the free-living nematodes have shown the possibility of rearing different species of *Rhabditis*, *Diplogaster*, *Cephalobus*, etc., in artificial media. The method employed by them consists in the use of watch-glasses, preferably of the "solid" kind, in which the nematodes are kept in drops of water to which is added a small quantity of the nutritive medium. To prevent evaporation of the medium the watch-glasses may be closed by glass covers fastened down with vaseline, or a number may be placed together, without covers, in a humid chamber. The latter alternative is employed in cases where the decomposition of the medium is so intense that the nematodes would succumb to the effect of the gases liberated in putrefaction, were they confined in the small space afforded by the cavity of a single watch-glass. By this method of culture nematodes can be maintained in conditions favourable to growth and reproduction, and, given a suitable medium, generation after generation can be reared

merely by transferring a mature female of one generation into a fresh watch-glass, in which it may become the parent of a further generation.

The temperature at which the cultures were carried on was the ordinary temperature of the laboratory.

For the liquid in the watch-glasses into which the nematodes were put ordinary tap-water or salt solution was used. It was first filtered to remove any nematodes which might be present in it. The culture medium was then added, and replenished afterwards from time to time as required.

If, as often happened, the medium in the watch-glass became too cloudy to allow direct examination of the nematodes under the microscope, they had to be removed singly on the point of a needle or a few at a time by means of a fine pipette, and transferred to a drop of water on a slide. Nematodes lying in water or in the nutritive medium in a watch-glass can be examined under the microscope with a low, but not with a high power. For examination with the high power they must be transferred to a slide.

Various media have been employed. They are here given roughly in the order in which they were tried. Most of them were abandoned as useless, and none have proved altogether satisfactory. The nematodes inhabiting the worm were found exceedingly difficult to rear. From some cause not yet understood, whole cultures have died off quite suddenly and unexpectedly, breaking the continuity of experiments and rendering it in many cases impossible to obtain conclusive results. The explanation for this may perhaps lie in the peculiar conditions in which these nematodes live. But, in addition to the sudden and unaccountable deaths of whole broods of nematodes in cultures which began promisingly, great difficulty was frequently experienced in the starting of cultures. Again and again every one of the larvæ removed from the nephridium died as soon as introduced into the food-medium. The difficulty of maintaining the cultures in a healthy state was on the whole not so great as that of making a successful start.



A nearly saturated solution of peptone (Witté's) was tried but this strength proved useless, as the nematodes died soon after being placed in it. Weaker strengths were then tried, with the same results, until a 15 per cent. solution was reached. This, though exceedingly dilute, proved satisfactory for one series of cultures. But after that peptone was almost uniformly unsuccessful and was abandoned.

Hay infusion (1 per cent.), like peptone, was successful for one series of cultures, but in other cases was unsatisfactory. Hay infusion, first sterilised and then inoculated with soil bacteria, was no improvement. A solution of urea was tried but was found to be useless. Meat extract and decaying meat were also unsatisfactory. Since these artificial media were unsuccessful, the natural food of the nematodes was used instead.

Earthworms decaying in damp soil were tried, as being the natural medium in which *Rh. pellio* develops. The mode of procedure is as follows. A freshly killed *Lumb. terrestris* containing nematodes in the nephridia and coelom is opened, and its gut is removed in order to obviate any chances of contamination with soil nematodes which may happen to be passing through in the soil which has been swallowed. The nephridia and the brown bodies in the coelom are left remaining. The worm is placed with soil in a watch-glass and then moistened with water. The soil is first heated to kill off any soil nematodes in it. But the temperature required to make certain of having done so probably renders it sterile and therefore valueless. At any rate, I find that it can be dispensed with. Within a week the decaying worm is seen to be covered with a whitish mass of actively writhing nematodes. Examination shows these to be adult males and females, the latter being the larger. Several generations are produced before the nutriment provided by the rotting carcase of the worm is exhausted. On comparing these adults obtained from the putrefying worm with those reared in peptone or hay infusion the difference in size is seen to be very striking (Pl. 37, figs. 2 and 3). The putrefaction

form is considerably the larger and stouter. Further, its reproductive organs are larger than those of the peptone form in proportion to the rest of the body, and the eggs in the uteri of the females are much more numerous, although, strangely enough, they are individually smaller. Decaying earthworm is of higher nutritive value than peptone, but it is in several respects less useful as a food-medium. Unless specially treated beforehand it cannot be employed for nematodes which are required to be reared in isolation, for the body-wall already teems with nematodes from the nephridia and coelom. In order, therefore, to remove these I proceed as follows.

A *Lumb. terrestris* is killed, and from along the whole length of the worm is cut a narrow strip of the dorsal part of the body-wall, a region to which the nephridia, infested as they are with nematodes, do not usually extend. To insure the entire removal of the nematodes imbedded in, or encysted on, the body-wall or present in any nephridia that may be left attached to the strip, the greatest care is exercised. The strip is laid on a slide and kept moist with water for about two days. During this period it is examined under the microscope from time to time. The nematodes which are present on the strip of body-wall congregate in the water round it, the encysted ones emerging from their cysts as it begins to putrefy. Unless the strip is heavily infested they can almost all be removed on the point of a fine needle. The relative thinness of the body-wall allows a fairly minute examination of it as a semi-transparent object, so that nematodes still left buried in the muscular tissue can be dug out with a needle, unless too deeply imbedded. When they have all been removed the strip is ready for use. A piece is cut from it and placed in a watch-glass with a small quantity of water and the nematode which it is required to cultivate. A control, consisting of another piece of the same strip moistened with water, is kept in order to make certain of the entire absence of nematodes from the medium employed. Decay is rapid, and the nematodes in the culture, like those reared on the body-wall decaying in its entirety, attain large dimen-

sions. While sharing with the latter medium this advantage over peptone, the new form of medium has also certain drawbacks in common with it. The body-wall, as it becomes more and more opaque in the course of decay, makes a dark background on which it is difficult to distinguish the nematodes in the culture. Occasionally, also, the whole contents of the culture develop an evil odour or in addition become clouded over with a dense scum, not only rendering examination impossible, but also causing the death of all the nematodes. But the chief drawback, and the one which rendered it necessary to discontinue the use of the strip method, was the presence of nematodes which were found infesting the medium in certain cases even after the exercise of the utmost care in attempting to remove them all.

A third medium, designed to avoid the disadvantages of its predecessors, has been devised and is prepared as follows :

Several *Lumb. terrestris* are killed and then cleaned by the removal of the gut. They are put with some water into a test-tube, which is then plugged with cotton-wool, heated in a steamer and kept at boiling-point for about two hours. The opaque broth is decanted and filtered. The almost clear resultant liquor, in which putrefaction very soon commences, is inoculated with the bacteria, which in the natural state are associated with the decay of the worm in the soil. For this purpose a freshly killed *Lumb. terrestris* is allowed to decay in a small quantity of water to which a little earth is added, and a spot of this fluid, carefully examined under the microscope to get rid of any nematodes in it, is added to the clear liquor before the bacteria from the air have had time to multiply in it. The liquor soon becomes cloudy with the growth of the soil bacteria and is then ready for use. This worm extract has been used for all the most recent cultures. It forms on the whole an excellent food-medium. Certainly it is the best that has yet been devised. Like the body-wall medium, it is more nutritious than peptone, and produces adults of large size. Over decaying body-wall it possesses the twofold advantage (for the sake of which it was devised)

of not being liable to contamination, and, of affording a fairly clear view under the microscope of the nematodes under cultivation. It is open, however, to the same objection as the body-wall medium. Occasionally an evil odour develops, accompanied as a rule by the appearance of an opaque scum, and all the nematodes in the culture die off.

The dimensions to which the adult nematodes attain vary according to the nutritive quality of the media on which they are reared, their size being directly dependent on nutrition. Thus those fed on worm extract or putrefying worm (the natural food) considerably outgrow those fed on peptone, and the latter cannot be regarded as typical of the species in regard to size.

The length of life of these nematodes in a natural state will be considered later in connection with their life-history. In artificial cultures, the period which the larva occupies in reaching maturity after its removal in the nephridial stage from the worm corresponds of course to that part of the nematode's life which in a state of nature elapses between the death of its host in the soil and its appearance as an adult. During this period growth is exceedingly rapid. In worm extract maturity is usually reached in four days. But occasionally mature females have been obtained in three days and males in two. Decaying body-wall takes about four days, peptone about six. Another period, easily ascertained in cultures, is that which elapses between the hatching of embryos from the eggs of successive generations of nematodes. In worm extract this is about eight days, in peptone about ten. While the period of growth prior to reproduction is fairly uniform, the length of life of the adults kept in cultures after reproduction is very variable. Some females succumb to the effort of reproduction, others survive the process by only a few days, and others live on afterwards for two or three weeks.

The use of an artificial culture medium shows that the distinction between oviparity and viviparity as specific characters does not exist in these nematodes of the earth-worm. They are oviparous or viviparous according to the food supply. Those

fed on peptone are always oviparous. Those fed on putrefying worm are usually oviparous at first, but, since they produce a far larger stock of eggs than the peptone form, they become viviparous later, when the eggs are being discharged into the uterus faster than they can be laid. The embryos are ready to emerge before the eggs have been laid, and therefore they hatch out in the uterus, and, when discharged from the parent's body, are no longer enclosed in the egg-shell. The nematodes of the earthworm are thus oviparous or viviparous according to the nutritive quality of the food supply. But both the peptone form and the putrefaction form behave, so to speak, viviparously if they die before all their eggs are laid, for the fertilised eggs that remain in the uterus, being quite independent of the parent for their nourishment, continue their development, and the embryos hatch out within the uterus of the parent, in whose decaying body they thrive and grow until nothing remains but a thin investment of cuticle.

#### IDENTIFICATION.

The encysted larvæ from the cœlom are reared to the adult stage (at which they can be identified) in the following manner. A number of the brown bodies from the tail of a freshly killed *Lumb. terrestris* are placed in a watch-glass with a little water. Disintegration and decay set in very soon, providing a natural food medium for the nematodes which emerge from the cysts. They grow rapidly, and within a week have developed into sexually mature adults of *Rhabditis pellio*, exactly similar to those obtained from the worm decaying in its entirety (Pl. 37, figs. 1 and 2).

This, however, is merely a confirmation of the results obtained by the early investigators. The question which the literature of the subject does not appear to have hitherto answered is, whether the active larvæ in the nephridia are the same species as these or not? I have not discovered that any previous worker has ever removed the larvæ from the nephridia and reared them to maturity in order to establish

their identity, and therefore I have done this myself. Larvæ from the nephridia of a *Lumb. terrestris* were put in a watch-glass with small strips of decaying body-wall and a little water. Growth was rapid. When sexually mature they were examined and were found to be *Rhabditis pellio*.

The nephridial and cœlomic larvæ, therefore, are the active and encysted forms respectively of one and the same species. This has been confirmed by all subsequent cultures. Further, I have found no other species but this inhabiting the living worm.

#### CONFUSION OF TWO SPECIES UNDER ONE NAME.

A perusal of the literature on *Rhabditis pellio* leads one to suppose that all the nematodes which have been encountered in connection with the earthworm and described under this name are genuine inhabitants of the healthy worm and belong to a single species. But whether this is really so, and whether the name does not require to be more clearly defined, are questions which I propose to discuss.

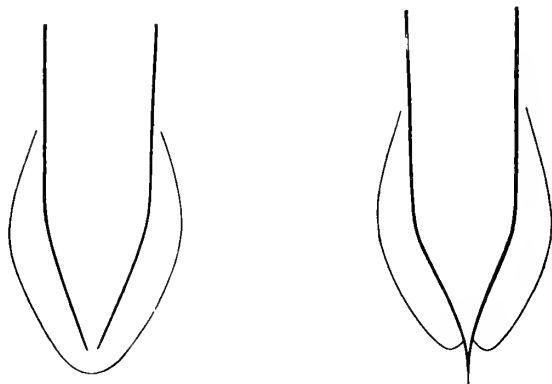
I have mentioned earlier in this paper in the survey of the literature that Bütschli (6) noticed certain differences between the adult individuals of *Rh. pellio* which he examined and those which had been described by Anton Schneider (1). There were various minor discrepancies. But the most striking point of dissimilarity was that the tail of Schneider's males did not project beyond the edge of the bursa, while that of Bütschli's males was prolonged to a fine point a short distance beyond the margin, which was slightly notched at the place of emergence.

Bütschli regarded these differences as unimportant, and supposed that the two different forms of bursa merged into one another by imperceptible gradations. Subsequent references to *Rh. pellio* in the literature of the Nematoda are for the most part very meagre. With the exception of Maupas, the writers do not comment on the dissimilarity of the two forms. Maupas (10), however, twenty-six years after the

publication of Bütschli's work, wrote as follows: "Sous ce nom de *Rhabditis pellio* on confond deux espèces distinctes: 1° le type décrit par Schneider ('*Monographie der Nematoden*,' p. 154); 2° celui décrit par Bütschli ('*Beiträge zur Kenntniss der freilebenden Nematoden*,' p. 112); la première espèce est une forme pélodérienne, la seconde une forme leptodérienne. . . ." (Text-fig. 1.)

In support of this statement that the two forms are

TEXT-FIG. 1.



"Peloderian" tail.

"Leptoderian" tail.

distinct species, there is, I think, convincing evidence. Firstly, Maupas says: " . . malgré l'opinion contraire du Bütschli, je me suis convaincu que cette absence ou cette existence d'un prolongement caudal mâle constitue bien un excellent caractère distinctif. J'ai eu occasion d'observer des milliers d'individus des deux types, obtenus dans des cultures isolées, et jamais je n'ai vu ce caractère faire défaut." Secondly, neither Schneider nor Bütschli saw the form described by the other, and Bütschli does not say he ever saw the intermediate forms which he supposed existed. Thirdly, in my own case, I have never found any but Bütschli's "leptoderian" form. The "peloderian" form of



Schneider and the "leptoderian" form of Bütschli are clearly distinct and separate species.

Now Bütschli's "leptoderian" adults are the form developed from the larvæ living in the worm, as my own results have abundantly shown. Schneider's "peloderian" adults, on the other hand, were obtained from worms decaying in soil, and were not actually proved to have been developed from the larvæ inhabiting the worm. Further, while those of my cultures of decaying worms, in which all chance of contamination from soil larvæ has been excluded, have never yielded any but the leptoderian species, those, on the other hand, in which earthworms are allowed to decay in ordinary soil have, as von Erlanger also (9) has shown, yielded others besides the "leptoderian" species. Indeed, on one occasion, in examining some earth in which several *Lumb. terrestris* had died and decayed, I found a number of larval nematodes, from which, when reared in worm extract, I obtained a male with a "peloderian" bursa and the bursal papillæ disposed, as far as I can judge from a rough drawing made at the time, similarly to those of Schneider's form. These considerations afford strong evidence that Schneider's "peloderian" form was a soil-inhabiting species, attracted while larval to the decaying worm, on which it developed and matured. This would be quite in keeping with the behaviour of the free-living species of nematodes inhabiting the soil, studied by Maupas and Potts.

On this hypothesis the reason why Schneider made the mistake of supposing his "peloderian" form to have developed from the nematodes inhabiting the worm as larvæ is not far to seek. Lieberkühn had seen the larval nematodes in the worm develop on the decay of the latter into sexually mature adults (which, however, he did not describe), and Schneider would naturally suppose, since he did not actually make the experiment and prove the contrary, that the adults which he himself found on the decaying worm had likewise developed from these larvæ. In the second place, the reason why Bütschli failed to recognise that the form which he des-

cribed was a distinct species from that of Schneider was evidently because Schneider's form, like his own, had occurred on decaying worms, and he did not realise that the presence of soil allows chances of contamination with soil-inhabiting forms.

It may be asked why neither writer recorded the form described by the other. The explanation may be that Bütschli probably did not find his infected earthworms decaying in soil like Schneider's, but killed them himself and allowed them to decay in water, in which case the "peloderian" form would not be present; and that Schneider made his description from only a few individuals, amongst which none of the "leptoderian" species happened to be included.

With the notable exception of Maupas, later writers, as I said before, do not comment on the difference of the two forms. The probable reason was that they saw Bütschli's "leptoderian" form only, because soil was excluded from contact with the putrefying worms which they used, or they may have seen both forms but did not suspect them to be distinct species because they did not recognise that the soil is a source of contamination.<sup>1</sup>

In view, then, of the confusion, under the same name, of two undoubtedly distinct species found in the same situation in the adult state but derived probably from different larval habitats, I propose to distinguish between them by narrowing the application of the name. I propose to restrict the name *Rhabditis pellio* Schneider to the "peloderian" form described by Schneider, and provisionally to designate the

<sup>1</sup> Since writing the above I have been able to consult Örley's 'Die Rhabditiden und ihre medicinische Bedeutung,' Berlin, 1886 (14). The tail of the male *Rh. pellio* which he describes (p. 33) is of the "peloderian" type and closely resembles that of Schneider's form. But whether he made his description from adults bred, without risk of contamination with soil nematodes, from larvæ actually inhabiting the living worm, and not from adults obtained from dead worms allowed to decay on soil (his method of obtaining large numbers of nematodes) appears to be doubtful.

leptoderian form, described by Bütschli, but wrongly regarded by him as belonging to the same species, by the name *Rhabditis pellio* Bütschli, non Schneider. No doubt the correct course would be to call the "leptoderian" species by a new name, but until Schneider's "peloderian" species has been re-examined, I hesitate to take this logical step.

In reading this paper no confusion should, I think, be caused by this splitting of what has hitherto been regarded as one species. This paper deals exclusively with Bütschli's "leptoderian" form, which I call *Rhabditis pellio* Bütschli, non Schneider, the species whose larvæ, both free and encysted, infest the living worm, and it is this form which is intended when the abbreviation *Rh. pellio* B. is used.

#### ANATOMY.

It will be well at this point to say something of the anatomy of *Rhabditis pellio* B. since the descriptions given by Schneider, Bütschli and von Linstow are not quite complete.

The accompanying table of measurements is intended to show the inferiority in size of the adult reared on peptone to the typical form fed on decaying worm. The arrangement is based on the admirable system used by Manpas (15). The fractions express the relation between the length of the particular region and the total length of the body. The length given for the buccal cavity, however, is relative to the total length of the œsophagus, of which it is regarded as part. The measurements of the adults—subject as they are to great variation—have been taken as far as possible from the largest individuals obtained.

The cuticle is transparent, and marked by exceedingly delicate transverse striations difficult to distinguish except at the anterior and posterior ends.

The mouth (Pl. 37, fig. 6) is bordered by three lips, one dorsal and two ventral, each of which is divided by a shallow groove into two lobes. Each lobe bears a pair of fine short papillæ. The buccal cavity in side view appears to be cylin-

TABLE I.—Measurements.

	Adult female.		Adult male.	
	Typical form.	Peptone form.	Typical form.	Peptone form.
Length . . .	2000 $\mu$	1238 $\mu$	1781	900 $\mu$
Œsophagus . . .	226 = $\frac{1}{9}$	248 = $\frac{1}{5}$	209 = $\frac{1}{9}$	
Tail . . .	150 = $\frac{1}{13}$	143 = $\frac{1}{9}$	72 = $\frac{1}{25}$	64 = $\frac{1}{14}$
Vulva . . .	1019	638		
Diameter . . .	143 = $\frac{1}{14}$	86 = $\frac{1}{14}$	104 = $\frac{1}{17}$	50 = $\frac{1}{18}$
Buccal cavity . . .	24 = $\frac{1}{9}$		23 = $\frac{1}{9}$	
Spicules . . .			67	49
		Larva in Nephridium.	Egg.	
			Typical form.	Peptone form.
Length . . .	510 $\mu$		56 $\mu$	62 $\mu$
Diameter . . .	28 = $\frac{1}{18}$		37 = $\frac{1}{1.5}$	47 = $\frac{1}{1.3}$

drical. It is of equal width throughout, except at its posterior end, just in front of the point where it is continued into the œsophagus. There it narrows and then widens again, ending in a thickened edge. The œsophagus has two bulb-like swellings, an anterior elongated one and a posterior rounded one, the latter bearing a dental armature. A part of the anterior end of the œsophagus is reflexed forwards round the posterior three fourths of the buccal cavity. The intestine (Pl. 37, fig. 2) is a tube formed between two rows of large alternating cells containing large nuclei. Its dark appearance by transmitted light is due to the granules deposited in its walls. The lumen of the intestine, and also of the whole of the alimentary canal, is lined with chitin. The rectum

(Pl. 37, fig. 8) is exceedingly short and has a narrow cavity. It can be distended by means of muscular fibres passing to the body-wall. Its anterior end is surrounded by three unicellular glands, two lateral and one median dorsal. The anus is ventral, and appears to be a transverse slit situated at the base of a papilla.

The nerve collar (Pl. 37, fig. 6) surrounds the œsophagus between the anterior and the posterior bulb. It is inclined obliquely in an antero-ventral direction.

The excretory canals (Pl. 37, fig. 6), which are difficult to see, pursue a sinuous course in the body-wall along either side. From each canal a single downward branch descends towards the excretory pore. Bütschli (6, pl. x, fig. 59e) represents these as uniting before reaching it. The excretory pore is situated in a median ventral position a short distance behind the posterior œsophageal bulb.

The female is long and narrow and tapers gently towards either end.

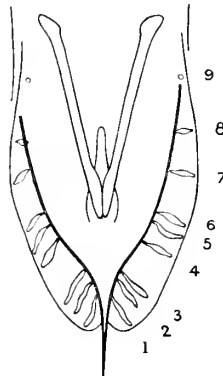
The tail of the female (Pl. 37, fig. 7) is prolonged to a fine point. It bears a pair of short papillae, one on each side, a little more than a third of the distance backwards from the anus. They do not project from the surface of the body, but are continued inwards, each as a delicate fibre running forwards towards the anus. They are not always exactly opposite one another.

The vulva, the reproductive aperture of the female, is placed slightly more than half-way along the body in a median ventral position, and has two projecting lips bordering a transverse slit. The vagina is short. In females of the typical form reared on putrefying worm the paired uteri are long and distended, and contain probably between one and two hundred eggs (Pl. 37, fig. 2). In the form fed on peptone they are smaller and contain five to twelve eggs (Pl. 37, fig. 3). The ovaries are long and reflexed dorsally, nearly reaching back to the vulva. The so-called "seminal receptacles," which are really the oviducts, are filled with spermatozoa, the female of this species reproducing as a hermaphrodite. They are short,

narrow tubes connecting the ovaries with the uteri and bent in the form of an **S** or a **U**. The eggs are oblong-oval. Those of the typical putrefaction form are  $56\ \mu$  in length and  $37\ \mu$  in diameter, while those of the form reared on peptone, though so much fewer in number, are  $62\ \mu$  in length and  $47\ \mu$  in diameter.

The bursa (Pl. 37, fig. 9), the copulatory organ of the male, is broadest in the middle. In all the males that I have seen the tail is prolonged a short distance beyond the end of

TEXT-FIG. 2.



Tail of a male *Rhabditis pellio* B. as seen in ventral view, showing the disposition of the bursal papillæ.

the bursa, whose edge is emarginated at the place of emergence. There are nine papillæ, disposed in groups of three each (Text-fig. 2). The posterior three are close together. Counting from the posterior end the second papilla is usually nearer to the third than to the first. The median three are also close together, and separated from the posterior group by an interval. In the median group the fifth papilla is usually nearer to the sixth than to the fourth. The interval between the anterior and median groups is equal to that between the median and posterior groups. In the anterior group there is a somewhat wide interval between the seventh and eighth, and a very wide interval between the eighth and ninth papillæ.

There is a great deal of variation in the disposition of the bursal papillæ in different individuals, even of the same brood. Single papillæ may be missing, extra ones may be present, or the usual intervals between papillæ may be altered. These variations may occur on one side of the bursa only, or on both. The fifth papilla may be nearer the fourth than the sixth, or the intervals between the fourth, fifth and sixth, or between the seventh, eighth and ninth may all be equal.

The papillæ do not reach the edge of the bursa but bend round, their ends pointing perpendicularly away from its ventral surface. The copulatory spicules (Pl. 37, fig. 9) are strong, slightly curved and thickened at their anterior ends. They are structurally independent of each other but work in unison. The accessory piece (Pl. 37, fig. 10) is a little more than half the length of the spicules. The testis (Pl. 37, fig. 1) is full of spermatozoa in different stages of development. The vas deferens is a tube formed of large cells with small nuclei. The lumen is narrow. The spermatozoa are oval and granular with a refractive nucleus of irregular outline.

#### QUESTIONS OF SEX.

##### (1) Analysis and Character of Reproduction.

The work of Manpas (14), supplemented by that of Potts (15), has shown that in *Rhabditis* and several closely related genera the mode of reproduction is by no means uniform. At first it was thought that all species were bisexual. It is now known that side by side with the bisexual species, in which males and females are produced in equal numbers, hermaphrodite and parthenogenetic species also exist.

In the hermaphrodite species, which appear to be even more numerous than the bisexual species, the females reproduce as self-fertilising protandrous hermaphrodites and constitute the bulk of the individuals, as a rule vastly outnumbering the males, which in some species are almost entirely wanting. The males, though apparently as perfectly



developed as those of the bisexual species, yet take no part in the reproductive process or only "re-fertilise" the females on rare occasions when the latter have exhausted the stock of spermatozoa produced in their genital organs. It would appear quite permissible to apply the term "female" to the reproductive individuals of the hermaphrodite species. They closely resemble the true females of the bisexual species of the same genera, the only essential anatomical difference being the presence of spermatozoa developed in the oviducts, which are hence called the "seminal receptacles." Indeed, there is evidence that the conversion of females into hermaphrodites is a process which is going on at the present day.

In the parthenogenetic species the females do not develop spermatozoa, and males are entirely absent.

So far as the question of its reproduction has received any attention at all, *Rhabditis pellio* appears to have been hitherto regarded as a purely bisexual form. But whether this is the case and whether the species is not somewhat variable in its sexual character are questions on which I hope the following investigation will throw some light. The males have never at any time been seen to take part in the reproductive process or even to exhibit any sexual instincts whatever, nor are they numerically equal to the females, as in the bisexual species.

When dead earthworms decay, the male and female nematodes which develop from the larvæ in the nephridia and cœlom propagate readily. But when larvæ were removed from a freshly-killed worm and placed in an artificial medium, the proportion of productive adults was in most cases considerably smaller, the medium being for some reason less favourable to reproduction than the natural food. This must be borne in mind in considering the following cultural experiments :

Culture A.—Two sexually mature females, which may or may not have been already fertilised by males, were removed from some decaying brown bodies of a *Lumb. terrestris* and isolated in worm-extract in watch-glasses. Both pro-

duced young. Both broods grew up into females (=  $F_1$  generation). No males were produced. One young female was isolated in worm-extract while still larval. It matured and reproduced, but both it and its offspring died immediately afterwards. In this culture the isolated female of the  $F_1$  generation unquestionably reproduced without being fertilised by a male, for not only were there no males in the brood from which it was taken, but it was still larval when isolated.

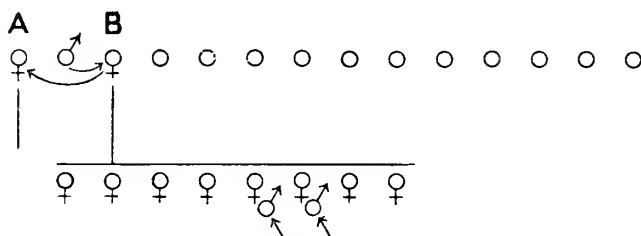
Culture B.—Two larvæ were removed from a nephridium and isolated in watch-glasses with peptone. Both developed into females, which matured and propagated. A series of about eight generations was produced. (The exact number was not recorded.) Amongst all the offspring of the various generations no males were found. The females of this culture, therefore, like the isolated female of the first filial generation of Culture A, propagated without any males being present to fertilise them. Spermatozoa were distinguished in the "seminal receptacles." Parthenogenesis being thus ruled out, reproduction must have been hermaphrodite.

Culture C.—Several nephridia were removed, together with the larval nematodes which they contained, from a freshly killed *L. terrestris* into a watch-glass with a little water. As the nephridia decayed the larvæ rapidly matured and reproduced. An  $F_1$  and an  $F_2$  generation were produced, and both were cultivated in worm-extract. In this culture, males, besides being present in the parental generation, were also produced in each of the filial generations. There were no cases of undoubted hermaphrodite reproduction, that is, in no instance did females which had been isolated while larval propagate offspring. Only those females which were kept with males reproduced. This is strong evidence for bisexuality, but is not decisive, since the females placed with the males may not have been fertilised by them, but may have produced their own sperm as hermaphrodites, and the reason why the isolated females refused to reproduce may have been, not the absence of males, but the poorness of the food-medium. This objection receives support from

the fact that, although only those females which were placed with males reproduced, yet by no means all, but only a small percentage of them, did so.

Culture D.—Several nephridia, with the nematodes which they contained, were removed wholesale from a freshly killed *Lumb. terrestris*. Fourteen of the larvæ were picked out and isolated in watch-glasses with worm extract. All but three died while larval, before their sex was indicated. (I represent such individuals in the culture-table by a plain circle. Of the three survivors two developed into females and one into a male. The one female, which I shall call A, began to lay disintegrating eggs. (Disintegration is a sign that the egg has

TABLE 2.—CULTURE D.



not been fertilised.) The male was put with the other female, B, which had not yet laid any eggs at all. Next day B began to lay fertilised eggs, from which larvæ ( $F_1$  generation) hatched out. The male was then removed from B and placed with A, which had by this time ceased to lay even sterile eggs. Two days later A also began to lay fertilised eggs, from which larvæ ( $F_1$  generation) hatched out.

Eight larvæ were isolated from among the offspring of B. All developed into females. So did all the isolated larvæ of the same brood and also A's brood. None of the eight isolated females reproduced, even though extraneous males, taken from a culture of the nephridial form reared on decaying nephridia, were placed with two of them. Nor did the isolated females of A's or B's broods reproduce.

The behaviour of the parental generation in this culture affords the strongest evidence for bisexuality that I have yet been able to obtain. The evidence is not absolutely conclusive, since the males have not actually been seen to fertilise the females either in this case or in any other, but it is very strong. The refusal of the isolated females of the  $F_1$  generation to reproduce is additional evidence. But, on the other hand, the two females of this generation with which the extraneous males were placed still remained unproductive.

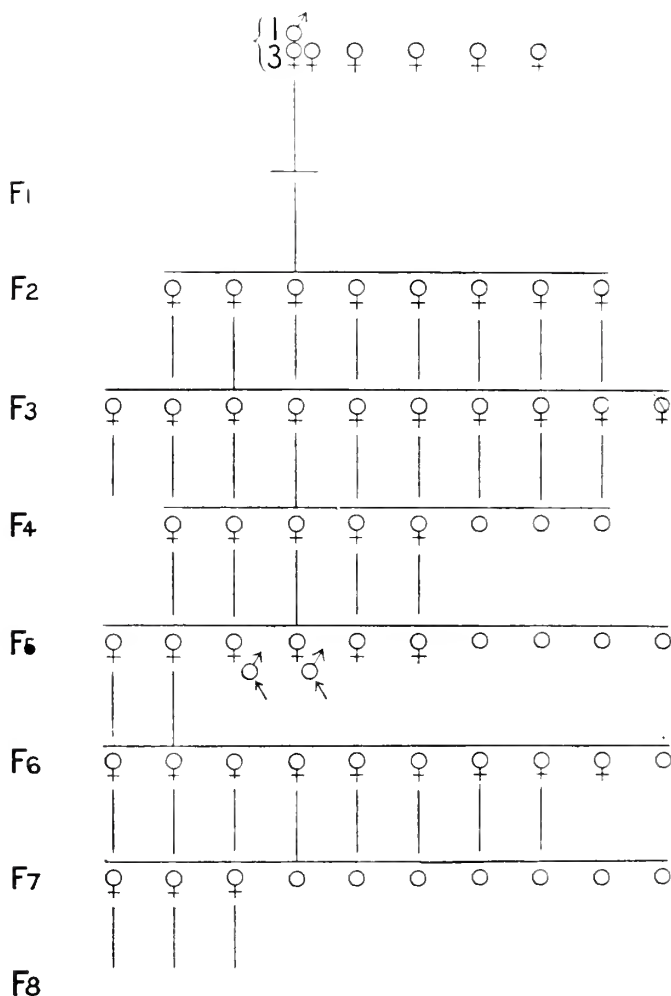
It should be noted that, if the male did in this culture actually take part in reproduction, it would appear that a male is capable of fertilising more than one female of the same generation.

Culture E.—Eight larvæ were taken from the nephridia of a freshly killed *Lumb. terrestris* and isolated in worm extract in watch-glasses. Seven developed into females and one into a male. Four of the seven females were kept isolated, and the male was placed with the remaining three females. On examining the cultures after an unavoidable absence, I found that, while the four isolated females had laid only unfertilised eggs (which rapidly disintegrated), the three females placed with the male had produced an  $F_1$  generation, which in its turn had given rise to an  $F_2$  generation. The parents and the first and second filial generations were all together in the same watch-glass, but could be distinguished by their different sizes. The  $F_2$  generation was larval and quite small. In the  $F_1$  generation I could see no males. The only male to be seen was the parental individual. Those females of the  $F_1$  brood, therefore, which reproduced probably did so as hermaphrodites, unless fertilised by the parental male, which I do not think probable.

Eight of the small larvæ belonging to the  $F_2$  generation were isolated. All developed into females, nor were any males seen among the unisolated remainder. Each of these eight females produced an  $F_3$  generation. Their mode of reproduction was unquestionably hermaphrodite, for they were isolated while in the larval condition.

Ten larvæ of the  $F_3$  generation were isolated from one of the most prosperous of these eight broods. All grew up into

TABLE 3.—CULTURE E.



females, nor did I see any males among the un-isolated remainder. All the females reproduced except one, which

died before it could reproduce. I represent such an individual by the female sign with its circle crossed by a line, to distinguish it from those which live long enough to reproduce but are for some other reason unproductive.

The culture was continued on the same lines until it died out in the  $F_3$  generation.

Regarding the isolated unproductive females as forming a control, the parental generation in this culture appear to have reproduced bisexually. The  $F_1$  generation reproduced probably as hermaphrodites. The  $F_2$  and all subsequent generations reproduced unquestionably as hermaphrodites. No males were produced in any of the broods. Extraneous males, taken from a culture of the nephridial form reared on decaying nephridia, were placed with two unproductive females of the  $F_3$  generation, but did not induce reproduction.

Culture F.—Ten larvæ were taken from the nephridia of a freshly killed *Lumb. terrestris* and isolated in worm extract in watch-glasses. Three died while larval. Of the remaining seven six developed into females and one into a male. The male and one female were killed accidentally. Of the other five females three reproduced but two did not, although an extraneous male taken from a culture of the nephridial form in decaying nephridia was placed with one of the latter.

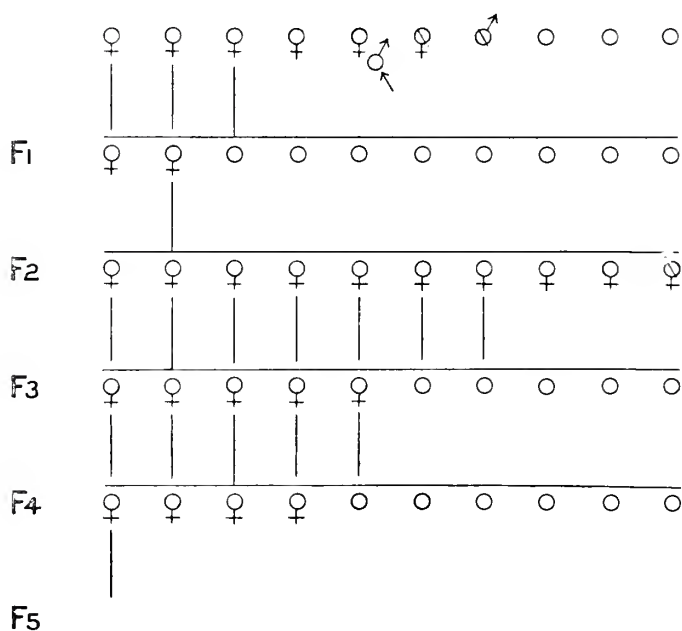
Ten larvæ of the  $F_1$  generation were isolated from one of the three broods. Eight died while larval. The remaining two grew up into females, nor did I see any males among the unisolated remainder. One of the two females reproduced, the other did not. The culture was continued on the same lines until it died out in the  $F_3$  generation.

In this culture reproduction was hermaphrodite in all cases. Nor were males seen in any of the broods. Further, this is the only case in which reproduction has been unquestionably hermaphrodite in the parental generation.

Conclusions.—From the numerous cases of reproduction by the females in the absence of males and the finding of spermatozoa in the genital organs of such individuals, it is

clear that the females are often—if not always—hermaphrodite. On the other hand, in spite of the fact that males have never been observed to exhibit any sexual instincts whatever, it seems probable, from the cases, especially in the parental generation, in which the females have propagated only when males have been present, that reproduction is sometimes bisexual. This

TABLE 4.—CULTURE F.



would mean that a number of true females exist side by side with the hermaphrodites as in *Rh. marionis*, a species described by Maupas (14, p. 506).

*Rh. marionis* affords also a remarkable instance of incipient hermaphroditism. Among the hermaphrodites not only are true females occasionally found, but also individuals which are partly hermaphrodite and partly female, the one half of the paired genital gland producing both eggs and



sperm, the other half giving rise to eggs only. The males also occasionally "re-fertilise" hermaphrodites whose stock of spermatozoa has become exhausted. In the present species, however, I did not see any of these partially hermaphrodite individuals or discover any cases of re-fertilisation.

It may be asked why bisexuality does not, like hermaphroditism, admit of experimental proof, without actual observation of the males in copulation with the females. The reason is that the results of an experiment may be misleading. A female placed with a male may reproduce, whilst a female, isolated when larval as a control, may not. This result might at first be regarded as an almost undoubted case of reproduction. But the real facts may be that the female placed with the male reproduced as a hermaphrodite, developing spermatozoa for itself and not receiving them from the male, in spite of appearances to the contrary, while the isolated female refused to reproduce, not because of the absence of males, but from some other cause. It should be mentioned that in this species, on account of the position of the gut, it is often very difficult, especially in young or unproductive females, to distinguish whether spermatozoa are present in the reproductive organs or not.

## (2) The Sex Ratio.

In regard to the sex ratio the results of the foregoing cultures and other observations not recorded here work out as follows. The nematodes removed from the freshly killed worm and reared in cultures, or obtained from the dead worm decaying under natural conditions, develop into males and females. Subsequent generations, however, bred under cultural conditions consist in almost all cases of females only. This difference between the ratio of males to females in the parental and in the filial generations under cultural conditions is most striking. In the parental generation the ratio of males to females was about 1 to 5, which for a hermaphrodite species is very high. After this the ratio was, with few exceptions,

so low that in the restricted number of generations through which the cultures lasted no males were seen at all. In a few instances, however, males did occur in the filial generations, and in such cases the ratio was sometimes high. The first filial generation of culture C, which consisted of 36 males and 31 females, furnishes a very striking instance of this.

Potts (15), in reference to the free-living hermaphrodite species which he studied, states that the production of males is cyclical, but that there is no rule that they should appear at stated intervals or restricting their production to a period or periods of maturity. My own cultures did not last long enough to enable me to throw light on the question. But, as has been already stated, although males are as a rule entirely wanting in the filial generations, they always develop in a high ratio in the parental generation consisting of larvæ taken from the freshly killed worm; and it is not unreasonable to suppose that, had the cultural series lasted over a longer period, males would sooner or later have reappeared.

Potts states that it is not probable that the sex proportions are governed by nutrition, and my own results bear out this opinion.

Maupas (14), in inducing males of *Rh. elegans* (p. 477) to re-fertilise hermaphrodites whose stock of sperm was exhausted, obtained an enormous increase in the proportion of males to females in the offspring, so that the sexes became almost numerically balanced. In attempting the same with *Rh. marionis* (p. 506) he did not succeed in affecting the ratio at all.

In the present species re-fertilisation, like ordinary fertilisation, has not been observed, and in those cases of reproduction in which males may nevertheless have taken part the sex of the offspring may not have been affected, though, curiously enough, the only males produced (besides those of the parental generation, which occur regularly among the nematodes taken while larval from the worm) have been among the offspring resulting from these cases.

The culture series made in the course of this work were for

some not very apparent reason exceedingly short-lived. Had the conditions been more favourable, they could have been maintained through a much larger number of generations, and would have thrown more light on the sexual phenomena of the species.

#### LIFE-HISTORY.

##### (1) Investigation of Problems relating to the Life-Cycle.

It has been already shown that active and encysted larvæ of *Rhabditis pellio* B. infest the nephridia and the coelomic cavity of *Lumbricus terrestris* respectively. It is also known that these larvæ, on the death and decay of the host in damp earth, become sexually mature and reproduce. Beyond this, except for a few scattered details mentioned in the literature of the Nematoda, the course which events take in a natural state has not been known. It was in the hope of being able to elucidate some of the interesting questions which suggested themselves in connection with the life-history that certain experiments were undertaken. The difficulties were considerable. It was found exceedingly difficult to imitate experimentally the natural conditions under which the nematode and also the worm live. Both animals were at a disadvantage, and were often unable to develop in a normal manner or in many cases to grow or even to live at all. The results obtained from this investigation are, therefore, incomplete, and still leave room for further work.

(1) The Nematode not confined to the Body of the Worm during the Latter's Lifetime.—An experiment to determine whether the larval nematode ever leaves the worm while the latter is living was several times made. A *Lumb. terrestris* was first thoroughly washed in order to remove any soil-nematodes which might be adhering to the outer surface of its body, and was then placed with filtered water in a watch-glass. The tail of the

worm was kept out of the watch-glass, so that no soil-nematode, which might happen to have been swallowed with the earth passing through the alimentary canal, could pass out into the water by the anal aperture. In almost every case a few larval nematodes of the active nephridial kind were found in the water around the worm after two or three hours. From the elimination of the anus as a means of exit there is every probability that the nematodes, being the active form, came from the nephridia, escaping by the nephridiopores.

That nematodes originally obtained from an earthworm will, when placed in a small quantity of water with another worm, make every effort to enter the second worm by any available orifice has been demonstrated experimentally by de Ribaucourt (11, p. 297) in the case of a smaller earthworm, *Notogama fœtida*, the brandling. I have myself on one occasion seen two larval nematodes with their anterior ends buried in the external surface of the body of a small earthworm about 1.5 inches long. They were probably entering by the pores. That the pores are capable of affording a sufficiently wide passage is shown by the effect of a sudden application of chloroform vapour. The worm is made to discharge forcibly through its pores large quantities of cœlomic fluid containing not only amœbocytes, but also occasionally both active and encysted nematode larvæ.

It seems probable, therefore, that during the life of the earthworm the larval nematodes pass out and in again by the external apertures of the cœlomic cavity and spend longer or shorter periods in the soil or even enter a different worm.

In order to ascertain by direct means whether *Rh. pellio* can be actually found living a free life in the soil, samples of garden earth were examined from time to time. Larval nematodes which were discovered were removed, and attempts were made to rear them to maturity with a view to establishing their identity by means of the sexual characters. From some cause not yet understood the attempts proved unsuccessful and the nematodes died before reaching maturity.

Maupas also (10, p. 623) has only once succeeded in finding *Rh. pellio* in the soil.

(2) A Period of Independent Existence passed in the Soil after the Death and Decay of the Earthworm underground.—A *Lumb. terrestris* was killed and allowed to decay. When the nematodes which it contained had become sexually mature and begun to reproduce, the worm was transferred to a fairly shallow glass dish, four inches in diameter, filled with soil which had previously been heated to kill off any nematodes present in it. The decaying worm was coiled up in as small a space as possible and placed in the centre of the floor of the dish. The earth was spread around and over it to a depth of about one inch and then moistened. Water was afterwards added from time to time in order to keep the earth sufficiently moist to prevent the desiccation of the nematodes. About two weeks after the experiment was begun, the soil in all parts of the dish was found to be teeming with nematodes. The worm occupied only a small space below the surface of the soil in the centre of the dish. But as far away as almost at the very edge of the soil, where it tended to become dried up, the nematodes were plentiful. Careful examination indicated that their relative abundance in different parts of the soil in the dish was no longer determined by the desire to be near to the dead worm for the sake of the food afforded by its putrefying body.

Although there were probably some thousands of nematodes in the soil, not a single adult was seen away from the worm. All those that had wandered away into the surrounding soil were larvæ, offspring of the adults introduced with the worm, and had reached the same stage of growth as that of the larvæ found in the nephridia. Six months later they were alive and plentiful but had not grown. Shortly afterwards the earth was accidentally allowed to dry and almost all of them died. But as much as eight months later, i. e. fourteen months after the beginning of the experiment, one larva was still alive and had not grown any

further. I do not know whether at the commencement only one or more than one generation of offspring was produced, but the larvæ were no larger at the end of the experiment than when I first examined them shortly after the commencement.

The results of this experiment would seem to indicate that the offspring of the nematodes which mature and reproduce in a worm that dies and decays in the soil are able to wander away from their food-supply after (or even perhaps before) it is exhausted and pass a period of free existence in the earth, perhaps only until the earliest opportunity arrives of infecting a fresh worm. It appears that, before leaving the carcase of the worm, they have reached the stage of growth corresponding to that of the larvæ found in the nephridia, and while free in the soil do not under ordinary circumstances, outgrow this stage. I have shown, however, in the previous section the difficulty of finding the larvæ of *Rh. pellio* in the soil to prove this.

(3) No Alternate Host discovered.—Since earthworms fall a prey to moles, thrushes, blackbirds, rooks and many less highly organised animals, it was only to be expected that *Rh. pellio* would be met with in connection of one sort or another with them. The larval nematodes which are eaten with the worm might succumb to the action of the digestive juices or travel unharmed through the alimentary canal and out into the earth with the fæces or pass a period of their existence within the body, either remaining larval or attaining sexual maturity.

The alimentary canals of four moles were examined on different occasions. They had been feeding on earthworms amongst other things, as was evident from the presence of setæ and portions of nephridia among the contents of the stomach. Larval nematodes, both free and encysted, were found in the stomach and intestine. The cyst of one of the encysted individuals was open at the end, and the nematode inside it was seen to be alive. All the unencysted individuals were dead, except a few which appeared after two days among

the rectal contents of one of the moles. All the larvæ were similar in size and appearance to the larvæ of *Rh. pellio* except these last, which were stouter and rather blunter-tailed. I tried to rear these and the encysted larvæ to maturity for the purpose of ascertaining whether they were *Rh. pellio*, but I was unsuccessful. No adult nematodes were seen. It appears likely, then, that the larvæ of *Rh. pellio* which are in the earthworm when the latter is swallowed by the mole succumb to the action of the digestive juices in their passage down the alimentary canal, or, if they survive, do not remain and mature but escape with the excrement into the soil.

The droppings of several thrushes were examined while still moist, and were found to contain a large number of larval nematodes similar in appearance and size to the nephridial larvæ of *Rh. pellio*. All were dead. But death may have been due, not to the effects of the passage through the gut, but to the frosts which prevailed at the time when the faeces were deposited. Other freshly dropped faeces of a bird have been found to contain living larval nematodes, showing that nematodes can survive a passage through the gut.

Manpas (10, p. 624) says that the larvæ of *Rh. pellio* are plentiful in all the slugs around Algiers. Whether the slug is an alternate host to the earthworm is not clear. But the impression conveyed is that the nematode can live equally well in the body of either and may pass quite casually into one or the other.

In this connection it might be mentioned that several attempts were made to rear again to maturity on decaying body-wall and on "extract" of *Lumb. terrestris* the larvæ which were obtained in the experiment with the worm decaying in soil in the glass dish. But all failed. This might be taken as an indication that an alternate host is necessary, but it is more likely that failure was due to other causes.

It was not discovered, therefore, whether the life-cycle of *Rh. pellio* is divided into two periods, one spent in the earthworm and the other in an alternate host. But it



seems very probable that the mole, thrush and other animals which prey on earthworms act merely as carriers of the nematode.

(4) Transference of the Nematode from Worm to Worm within the Cocoon.—I endeavoured to discover whether the larvæ of *Rh. pellio* are ever transferred from the parent worm to the young worm inside the cocoon. The cocoons are often plentiful in the soil and can be procured by digging. But it is not easy to discover with certainty to which species of earthworm they belong, and I found it impossible, by digging in the earth, to obtain cocoons which I was sure belonged to *Lumb. terrestris*. An unsuccessful attempt was made to obtain cocoons belonging undoubtedly to *Lumb. terrestris* by keeping a number of mature worms of this species under natural conditions in the soil, but confined apart from all other species of earthworms.

I have, however, examined many cocoons which I found by direct search in garden soil, but without being able to ascertain to which species they belonged. They were of very varying sizes and belonged no doubt to worms of more than one species. Nematodes were found living within several of them in the albuminous fluid bathing the embryo worm. The nematodes were larval and resembled the nephridial form of *Rh. pellio*. I attempted to rear them to maturity in order to determine their identity, but was unsuccessful.

I also removed all the young worms that were ready to hatch from the cocoons, killed them and allowed them to decay in a little water. Had they been already infected while in the cocoon, the nematodes would have appeared when they decayed. But this did not happen.

More evidence is required on this question. Neither the identity of the cocoons examined nor of the nematodes found inside them was known. But it appears quite probable that the larvæ of *Rh. pellio* do not infect the embryonic *Lumb. terrestris* in the cocoon even though they may be present with it.

If the larval nematode found in the cocoon be *Rh. pellio*

it is not difficult to understand how it gets there. When the cocoon is being slipped forward towards the head of the worm it must pass over the nephridiopores, and it is quite easy to imagine a few nematodes escaping from the nephridia into the cocoon through these apertures.

(5) Occasions when the Larval Nematode attains Sexual Maturity.—The sexually mature adult of *Rhabditis pellio* is found engaged in reproduction in large numbers in the dead earthworm decaying in soil, as is shown in the case of worms which have died a natural death in the earth and are found there in a state of putrefaction, as well as by artificial methods. But I have never seen the adult or its eggs or newly hatched larvæ in the live earthworm, and I do not believe that they ever occur there.

Thinking that *Rh. pellio* might possibly attain the mature condition in the soil as well as in the dead worm, I have searched samples of earth for it. But I have failed to find it.

It is not, therefore, certain whether the larval *Rh. pellio* in the course of its development becomes sexually mature in ordinary soil or only in the dead worm. But if a larva which has passed out into the soil from the body of a live worm finds a quantity of nutritive substance such as the decaying carcase of some animal, it seems reasonable to suppose that under such circumstances it will mature and reproduce just as it would have done had it remained in the worm till the latter died. Such behaviour would correspond to that of the larvæ of the free-living soil-inhabiting species, which grow into adults and propagate when they find some putrefying substance in the soil.

(6) Mode of Infection of a Fresh Worm by the Larval Nematode.—From the fact that the adult nematode is entirely absent from the live earthworm it is evident that the infection of a fresh worm is carried out by the larva.

There are two possible ways of entrance into the body: (i) through the external apertures of the coelomic cavity, or (ii) by the mouth or anus into the gut and through the gut-wall.

(i) I have not cut sections of the gut of *Lumb. terrestris* to ascertain whether nematodes can be seen actually traversing the gut-wall, and I have no evidence on this question except the fact that on three occasions I have found species of larval nematodes alive among the soil contents of the gut. Whether the nematode is capable of making its way through the tissues of the gut-wall is not quite certain. The genus *Rhabditis* is not provided, like some genera, with piercing mouth-parts, nor do the nematodes in a nephridium removed from a freshly killed worm appear at all capable of migrating through even its relatively thin wall. At the same time they must be able, to some extent, to push their way in among the tissues of the worm to be found imbedded in the muscular layer of the body-wall, as is sometimes the case.

(ii) With regard to the other way of entrance, de Ribaucourt, as already shown (11, p. 297), has contributed definite evidence that nematodes can enter a worm by the coelomic pores. I have on one occasion seen larval nematodes apparently entering by the pores, and I have shown that the larvæ can leave the worm by the same means. K. C. Schneider (13, p. 423) believes that the nematodes in the bladder of the nephridium have wandered in through the nephridiopore.

It seems probable, therefore, that whether or not *Rh. pellio* in the natural state infects the worm through the gut-wall as well, it certainly does so by entering through the dorsal pores, nephridiopores or reproductive apertures, or possibly by all of these.

(7) Reason for the Presence of *Rh. pellio* in the Worm in Two Different Larval Conditions.—The most likely reason for the existence of two kinds of larvæ—free and active in the nephridia and seminal vesicles, encysted and quiescent in the coelomic cavity—accords with the supposition that the nematodes enter the worm by the pores.

(i) Firstly, those which pass in by the nephridiopores find themselves, as K. C. Schneider says, in the bladder-parts of the nephridia where they remain as the nephridial form.

Those, too, which find their way in by the spermiducal apertures and travel up the vasa deferentia are the same as are found on opening the seminal vesicles.

(ii) Secondly, those which enter by the dorsal pores and the oviducal apertures find themselves in the cœlomic cavity. Here they are attacked as foreign bodies by the amœboytes and encyst. Keng (8, p. 391) describes the way in which the amœbocytes surround and cover nematodes in the cœlom, hampering their movements by means of fine protoplasmic threads into which they can become drawn out. He gives a drawing (pl. v, fig. 44) of a nematode struggling with amœbocytes. When the nematode is completely covered it apparently sheds the outer layer of its cuticle. The amœbocytes soon die and turn brown. The cyst is composed of the loosened outer layer of cuticle with its investment of dead brown amœbocytes. The completely and partially encysted nematodes are gradually worked backwards through successive segments by the movements of the worm until they reach the tail, where, with cysts of *Monocystis* and discarded setæ, they are compacted by pressure into the flattened oval masses and cemented together by their investment of broken-down cœlomic corpuscles. The great majority of the nematodes found in the cœlomic cavity of a fresh-killed worm are encysted. But in addition to these there are some which are covered with amœbocytes but not completely enclosed by a cyst, and some which are quite free. Those that are found quite free have probably not yet been attacked by amœbocytes. Those that are covered with amœbocytes but are not fully encysted were probably about to become so when the worm was killed. The latter, being only slightly encumbered, are no doubt those which, disengaging themselves from the imprisoning lymph-cells, are the first to be seen escaping from the brown bodies. The only occasion that I know when the larvæ emerge from their cysts is on the death and decay of the worm, when the food supply becomes plentiful and nutritious. But only a certain proportion of the fully encysted larvæ do so. The remainder appear to have degenerated, for

they do not recover from the conditions of diminished vitality under which they have been existing. Those which emerge are therefore the more recently encysted ones, which have not yet begun to degenerate.

(8) Nature of Food.—*Rhabditis pellio* belongs to a genus, most of whose members are free-living in the soil and mature and reproduce on animal or vegetable substances in putrefaction. It resembles these in the conditions under which it matures and reproduces. But it differs from them in spending all or part of its larval period in the body-cavity of an animal instead of in the soil. It does not appear to do any damage to the worm. It occurs in the largest and healthiest-looking individuals in quite as large numbers as in weakly specimens. Moreover, having, like all the species of the genus *Rhabditis*, an unarmed buccal cavity, it is incapable of feeding on the tissues of the living worm.

Its food consists of liquids, which are taken in by the suctional action of the œsophagus. It grows most rapidly in media which are swarming with bacteria, which shows that it is upon the bacteria or the products of their action that it lives. What Potts says of the free-living species of the genera *Rhabditis* and *Diplogaster* applies to this species also. He says (15, p. 444): "It is only in the presence of great numbers of bacteria, or the substances formed by them, that the nematodes thrive so well. . . . It has not been discovered whether digestion takes place by the secretion of juices dissolving the protoplasm of the bacteria, or is merely confined to the absorption of soluble substances present in the culture fluid and prepared by the action of bacteria. . . . An easily observable phenomenon of nematodes in culture is the rapid pumping action of the second œsophageal bulb and the rectum, and it may be argued from this that the nutriment obtained from the stream of fluid so constantly passing through the alimentary canal is in the form of easily abstracted soluble substances. The insignificant development of glandular cells (which are found only in the œsophagus) may be cited against an intra-intestinal digestion of the bacteria, and,

whatever else its significance may be, the chitinous layer which lines the alimentary canal throughout must prevent an ingestion of bacteria by the endoderm cells themselves in such a way as *Colpidium* preys upon the bacteria of the soil."

The active nephridial larvæ feed on the bacteria or bacterial products which pass down the nephridial tube in the current of coelomic fluid, or which may congregate in the dilated bladder-part. But the number of bacteria in the living worm must be relatively small compared with the number produced on its decay, and there is, therefore, little or no growth in this condition. Hence the larval period is a long one. But on the death and decay of the worm large numbers of bacteria are produced, and, food now being plentiful, growth is rapid, and the nematodes mature and reproduce in a few days.

*Rhabditis pellio*, then, not only belongs to a genus most of whose species are free-living, but in all respects except its habitat appears to behave like one of the free-living species of that genus. The rôle which Maupas (10, p. 623) ascribes to it when he speaks of it as "locataire inoffensif" is the true one, and the advantages which it gains from its association with the worm are protection and dissemination.

## (2) Probable Course of the Life-Cycle.

The results obtained in the foregoing investigation are not conclusive, but they suggest, I think, that the life-history of *Rhabditis pellio* is somewhat as follows:

When an infested *Lumbricus terrestris* dies in moist earth, the larvæ of *Rh. pellio* which it contains feed on the nutriment afforded by its putrefying carcase, the encysted individuals emerging from their cysts. Growth is rapid. Within a week they develop into sexually mature males and females, and the latter reproduce. Reproduction is often—possibly always—hermaphrodite. But sometimes it seems to be bisexual. I am not certain whether in a natural state the body of the decaying worm is capable of affording sufficient nutriment for the production by these offspring, in their turn,

of a further generation. The young reach the same stage of growth as that of the nephridial larvæ. They then leave the carcase of the worm and wander into the soil. Here they live for a longer or shorter period but do not increase in size. Sooner or later they infect an earthworm, making their way in by the external apertures of the cœlom. Those that enter by the nephridiopores take up their position in the terminal, bladder-like part of the nephridia. Those that use the spermiducal apertures travel up the vasa deferentia and occupy the seminal vesicles. Lastly, those that pass in by the dorsal pores and the oviducal apertures find themselves in the cœlom, where, being attacked by the amœbocytes, they encyst. These encysted larvæ coated with amœbocytes are worked backwards by the movements of the worm till they come to rest in the tail end of the worm, where, together with other foreign bodies, such as cysts of *Monocystis* and discarded setæ, and with masses of dead brown-coloured amœbocytes, they are compressed and cemented into the brown bodies which are found there.

In this larval condition the nematodes remain during a protracted period without growth, the encysted form without movement, until on the death and decay of the worm in the soil they grow, mature and reproduce.

When infested worms are eaten by moles, thrushes or other predatory animals, it is probable that the nematodes travel down the alimentary canal, and, whether alive or dead, pass out with the fæces into the soil. I have not yet any evidence of the existence of an alternate host, within whose body the nematode spends a part of its life-history.

During its larval existence the active nephridial form probably passes out and in by the external apertures of the cœlom and spends longer or shorter periods in the soil. Or it may change hosts, entering and inhabiting another worm. But if, while in the soil, it finds some animal or vegetable substance in putrefaction, it may mature and reproduce on the spot, as it would have done in the worm had it remained till the death of the latter.



This passing backwards and forwards between the body of the worm and the soil may have interesting consequences when it takes place during the detachment of the cocoon of a worm engaged in reproduction. The finding of larval nematodes within the closed cocoon suggests that, when the latter is being slipped forwards towards the head, some of the nematodes in the nephridia pass out into the cocoon through the nephridiopores. But the young worm in the cocoon has not been found to be infected, and the inference is that the presence of the nematode is only accidental, and that, when the young worm hatches out of the cocoon, the nematode escapes into the soil.

#### SUMMARY OF PRINCIPAL RESULTS.

(1) The active larval nematodes inhabiting the nephridia of the common earthworm, *Lumbricus terrestris* Linn., and the encysted larvæ found in the cœlum of the same host belong to the same species of *Rhabditis*.

(2) This species I regard as distinct from *Rhabditis pellio* Schneider, with which it has hitherto been confused.

Bütschli first described it but regarded it as merely a form of *Rh. pellio*. *Rh. pellio* is, however, a soil species which may mature on decaying earthworms; the species described in this paper is a parasite of the earthworm and has not hitherto been recorded with certainty in the soil. I propose to designate it provisionally by the name *Rhabditis pellio* Bütschli, non Schneider.

(3) The dimensions attained by the adults of this species vary considerably according to the nutritive value of the culture medium employed. Decaying earthworm, the food material on which the species grows to sexual maturity in a natural state, has been found more nutritious than the usual artificial media, such as peptone, and promotes correspondingly greater growth.

(4) A medium consisting of extract of earthworms has

been devised, by which the natural food is conveyed in a nutritious form, convenient for cultural purposes.

(5) The nematodes removed from the freshly killed worm and reared in cultures, or obtained from the dead worm decaying under natural conditions, develop into males and females. Subsequent generations, however, bred under cultural conditions, consist in almost all cases of females only.

(6) Examination of these cultures, consisting of females only, reveals the fact that they are in reality hermaphrodite.

(7) Reproduction is frequently—perhaps always—hermaphroditic. But cases occur in which it appears to be bisexual—that is to say, both hermaphrodites and true females may exist side by side in the same species, as in *Rhabditis marionis* Maupas.

No “partial hermaphrodites” have been found, nor have any cases of “re-fertilisation” been observed.

(8) The numerical ratio of males to females is extraordinarily variable, and no rule governing the fluctuations has yet been found.

(9) I have not been able to follow out the life-history in its entirety, but evidence is afforded of the probable mode of transmission and of infection.

BIRMINGHAM,

November 26th, 1912.

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#### EXPLANATION OF PLATE 37.

Illustrating Mr. G. E. Johnson’s memoir “On the Nematodes of the Common Earthworm.”

*acc. p.* Accessory piece. *amœb.* Envelope of dead amœboocytes. *buc. cav.* Buccal cavity. *burs.* Bursa with bursal papillæ. *cy.* Cyst. *ex. can.* Excretory canal. *ex. po.* Excretory pore. *gen. rud.* Genital rudiment. *int.* Intestine. *nerv. col.* Nerve-collar. *œs. b.* Œsophageal

bulb. *ov.* Ovary with developing eggs. *ovd.* Oviduct or "seminal receptacle" with spermatozoa. *pap.* Papilla. *rect.* Rectum. *rect. gl.* Rectal glands. *sp.* Copulatory spicules. *test.* Testis with developing spermatozoa. *ut.* Uterus with fertilised eggs. *v. d.* Vas deferens. *vulv.* Vulva.

Fig. 1.—A mature male of *Rhabditis pellio* in latero-ventral view, showing the reproductive organs. ( $\times 90$ )

Fig. 2.—A mature hermaphrodite of *Rh. pellio* in lateral view, showing the reproductive organs. This is the typical form developed in, and nourished on, the decaying earthworm. ( $\times 90$ )

Fig. 3.—A mature hermaphrodite of *Rh. pellio* fed on peptone, showing its inferiority in size to the typical hermaphrodite nourished on the decaying earthworm, and the smaller number of eggs in the uterus. ( $\times 90$ )

Fig. 4.—An active larval individual of *Rh. pellio* from the nephridium, showing the genital rudiment. ( $\times 230$ )

Fig. 5.—An encysted larval individual of *Rh. pellio* from the coelom in the act of escaping from the cyst. ( $\times 230$ )

Fig. 6.—The anterior end of a hermaphrodite of *Rh. pellio* in lateral view, showing the mouth-parts, nerve-collar and excretory system. ( $\times 350$ )

Fig. 7.—The tail of a hermaphrodite of *Rh. pellio* in ventral view showing the rectal glands and caudal papillæ. ( $\times 350$ )

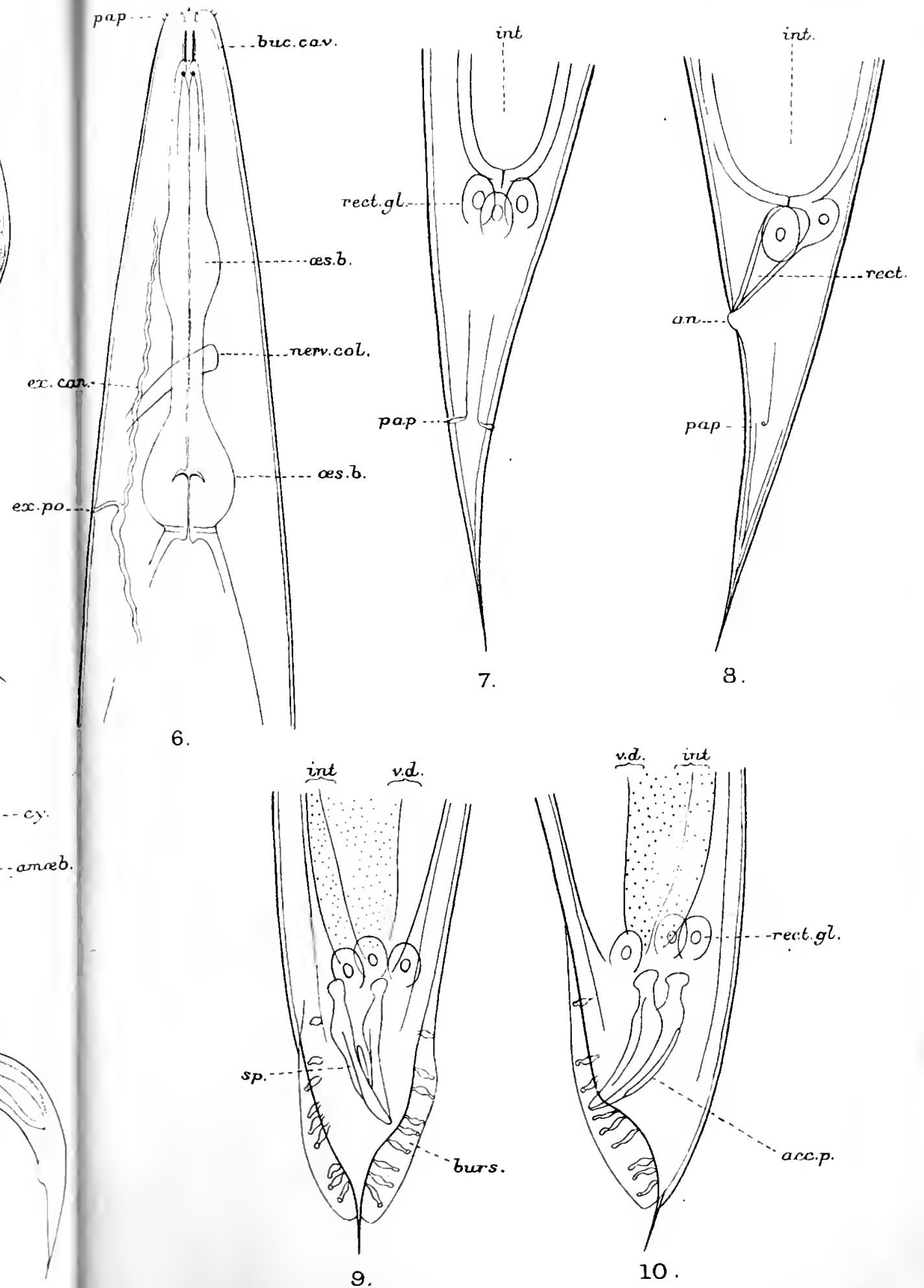
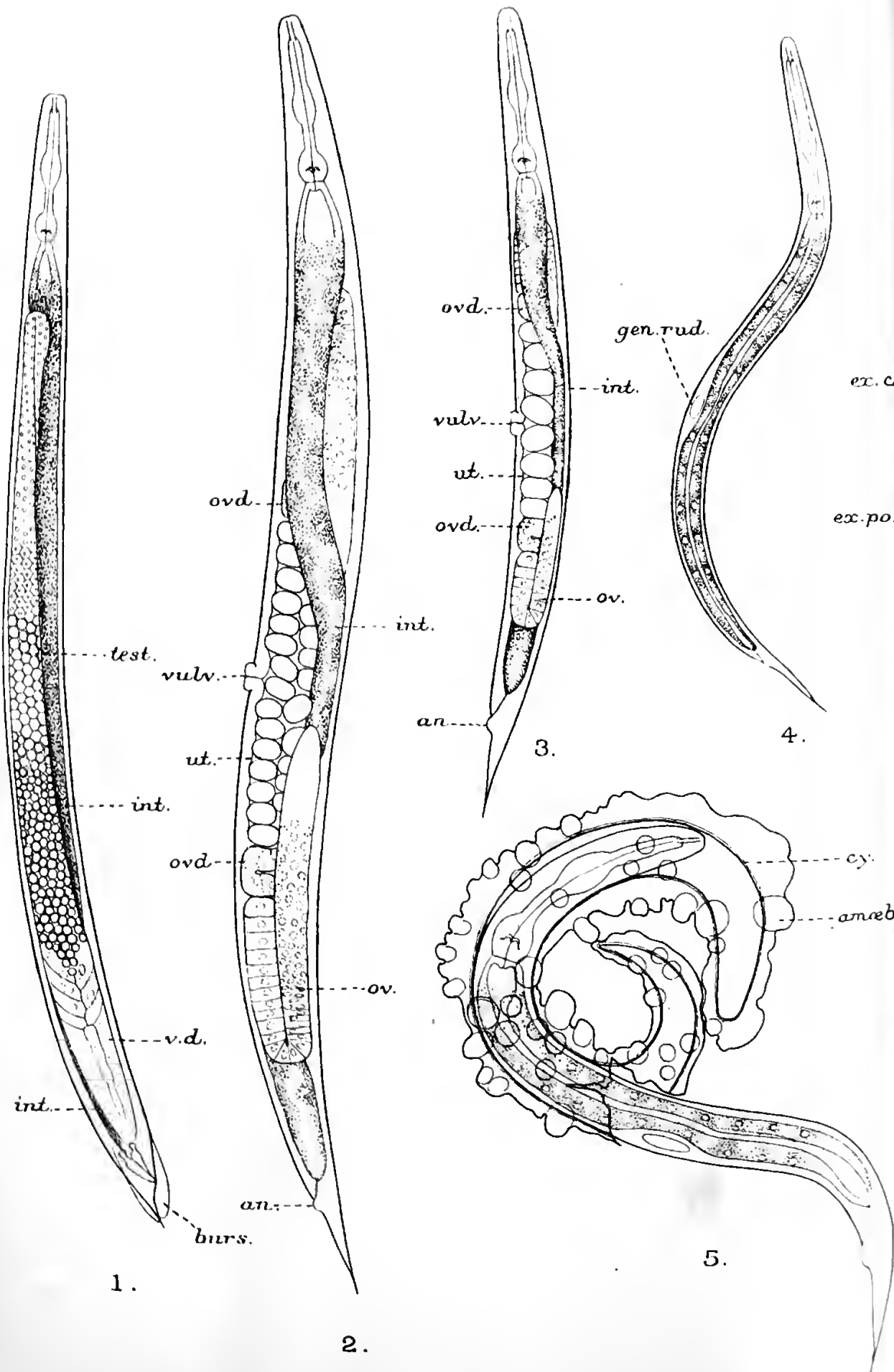
Fig. 8.—The tail of a hermaphrodite of *Rh. pellio* in lateral view, showing the short rectum in distension and the anus. ( $\times 350$ )

Fig. 9.—Tail of a mature male of *Rh. pellio* in latero-ventral view, showing the bursa, bursal papillæ and copulatory spicules. ( $\times 350$ )

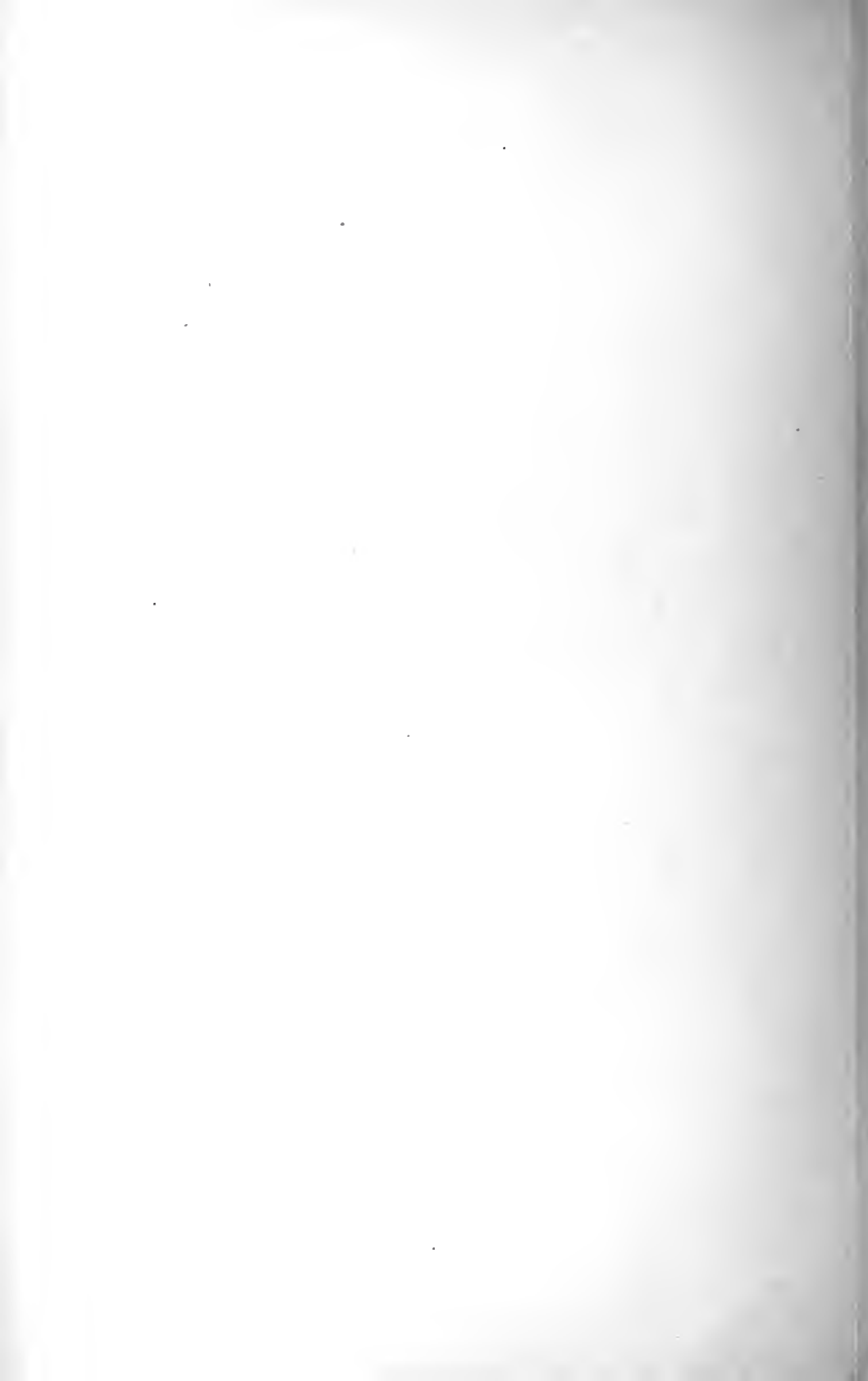
Fig. 10.—Tail of a male of *Rh. pellio* in dorso-lateral view showing the copulatory spicules and the accessory piece. ( $\times 350$ )











**The Structure and Biology of Schizoneura lanigera, Hausmann or Woolly Aphis of the Apple Tree.**

**Part I.—The Apterous Viviparous Female.**

By

**J. Davidson, M.Sc.**

(Liverpool).

(From the Cooper Laboratory for Economic Research, Watford, Herts.)

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With Plates 38-42, and Text-figs. A-D.

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I. INTRODUCTION.

THE wide distribution of *Schizoneura lanigera* and its importance as a serious pest to fruit-growers is generally recognised by workers on economic entomology, and so far as I am aware, no detailed account of the structure of this insect has been given before. It therefore seemed to me very desirable that a study of the anatomy of this species should be carried out.

In the present paper I propose to treat of the structure of the apterous viviparous female, and hope shortly to complete the study of the winged viviparous female and other stages, which will be the subject of the second part of this paper.

I desire to take this opportunity of expressing my sincere thanks to Mr. W. F. Cooper, who has most kindly given me every facility for carrying on this work, and to Mr. L. E. Robinson for the generous and kind way in which he has given me advice during its progress.

## II. TECHNIQUE AND METHODS.

In order to ensure a constant supply of living material, several young apple-trees, both in the laboratory orchard and in the green-house, were infested with *Schizoneura lanigera* and kept under close observation.

For the study of the chitinous exoskeleton, entire specimens were treated with cold 10 per cent. potash for several hours, and after being washed in distilled water to which a trace of acetic acid had been added, were dehydrated, stained in xylol saturated with picric acid and mounted in Canada balsam.

Chloral hydrate and phenol in the the proportion 2:1 proved a useful clearing agent, the insects being left in the mixture, which was kept warm until cleared. They were then transferred direct to xylol, stained with picric acid or orange G and mounted in Canada balsam.

For the study of the head and its endoskeleton the parts were dissected out and macerated in cold 10 per cent. potash, washed, and examined in glycerine. Glycerine jelly was also used as a mounting medium.

Dissections were carried out on living and preserved material under the Zeiss binocular microscope, the specimens being fixed down on a small wax plate beneath the examination medium, special parts being isolated and examined on a slide. Normal saline solution, glycerine, 70 per cent. alcohol and oil of cloves were used as examination media. The animals resist wetting very much, which proves troublesome when dissecting living specimens under normal saline solution. If the surface of the aphid is smeared with 70 per cent. alcohol after fixing on the wax plate, and before pouring on the salt solution, the difficulty is overcome.

Entire specimens were fixed with warm picro-sulphuric acid (Kleinenberg's) formula, and Carnoy's fluid, the latter fixative giving excellent results. Internal organs were dissected out in normal saline solution and fixed with Perenyi's fluid or corrosive sublimate.

For staining sections, hæmatoxylin (Ehrlich), methylene-blue, eosin and orange G have been used. Borax carmine was used for staining organs in bulk after corrosive sublimate fixation.

Material for sections was imbedded in paraffin wax melting at 58° C., for about fifty minutes to two hours, sections being cut 6 to 10  $\mu$  thick. Material imbedded in a lower melting-point wax (45° C.) gave poor results.

The drawings have been made with the aid of the Abbé camera lucida from dissections, special preparations of parts and serial sections through the body.

### III. SYSTEMATIC POSITION, LIFE-HISTORY, AND HABITS.

*Schizoneura lanigera* is a member of the order Hemiptera, belonging to the family Aphididæ, the members of which are popularly known as "green fly" or "plant lice."

It is classified in the group Schizoneurini, of the sub-family Pemphiginæ.<sup>1</sup> On account of the quantity of white waxy threads that individuals of this species produce from the dorsal wax-glands they are known as "woolly aphids," or "American blight." This latter term is, however, misleading, as this pest, according to Theobald (1897), is European in origin, and was no doubt imported into America with imported stock. Marlatt (1897, p. 2), on the other hand, considers that the evidence is in favour of its American origin and he refers to the fact that it was first observed in England in 1787 on some stock imported from

<sup>1</sup> The classification of the Aphididæ is at present in an unsettled state. Passerini (1863), in his 'Aphididæ Italica,' includes *Schizoneura* under the sub-family Pemphiginæ. Buckton (1875-82) separates the Pemphiginæ and Schizoneurinae as two distinct groups. Del Guercio (1900) classifies the genus *Schizoneura* in the group Myzoxylides, a division of the sub-family Myzoxylinae. Mordwilko (1908), classifies the genus *Schizoneura* (Hartig, 1841), in the group Schizoneurina, a division of the sub-family Pemphiginæ. Tullgren (1909), has adopted this position for the genus in his, 'Aphidologische Studien.'

America. Buckton (1880, p. 91) refers to Dr. Asa Fitch and Prof. Cyrus Thomas as refuting the idea of an American origin for woolly aphis, and also refers to Serville and Amyot as stating that it probably came to Europe through England, from America.

Owing to the transportation of nursery stock woolly aphis has been carried from one country to another, so that it is now established practically wherever the apple is cultivated.

Plant lice live on juices drawn from the tissues of growing plants, and in accordance with the sucking habits, the mouth parts, as is common amongst Hemiptera, are modified to form piercing and sucking tubes.

*Schizoneura lanigera* attacks practically all varieties of apple trees in Britain, producing galls on the roots and branches. It is very destructive to nursery stock and young trees. According to Theobald (1909, p. 144), who cites French (1904) and Lounsbury, apples grafted on certain stocks, particularly the Majentin and Northern Spy, do not suffer from the root form.

However, so much has been written about the habits of woolly aphis that it is, perhaps, unnecessary to say much on the point here.

Although the damage done by *S. lanigera* on the roots and shoots of apple trees, resulting in the formation of gall-like growths, is familiar to economic entomologists, there appears some doubt as to the exact way in which the galls are produced. Riley (1879), discussing this point with reference to *Phylloxera*, refers to the work of Maxime Cornu (1878), "*Études sur le Phylloxera vastatrix*," who accounts for the swellings caused on the vine by *P. vastatrix* as purely due to the piercing action of the mouth parts, and the subsequent absorption of the sap from the wounds thus formed. There are many aphids however, which, having fully developed mouth parts, do not cause galls to develop on the plant host. Some other factor must, therefore, be considered. As Riley ('*Science*', 1895, n.s., i, p. 457) points out in the case of the larvæ of gall-flies (*Cynipidæ*), it is very probable that in

*S. lanigera* some poisonous substance is secreted into the wound. Riley (1879) is inclined to the view that the salivary glands in *Phylloxera vastatrix* may produce the necessary irritating substance; but the same argument used above may be applied in this case, for well-developed salivary glands are present in aphids which do not produce galls. Grove (1909) has described salivary glands in *Siphonophora rosarum* which closely correspond to those of *Schizoneura lanigera*, yet the former species does not produce galls on the host plant. It may be however, that the histology of the salivary glands of those species which do not produce galls differs from the species which cause galls, and that in the latter case a special ferment is produced which is not present in the non-gall-producing species.

Blomfield (1906) has described in some detail the origin and structure of the cankerous growths produced by woolly aphids. From sections through diseased galls he has shown that the effect is due to some undue influence acting on the cambium cells. He considers that the factor of mechanical irritation is not the important one, and suggests that a ferment substance is possibly produced by the salivary glands, but he failed to establish proof of this suggestion. Künckel (1867, p. 45), who inoculated plants with the extract of salivary glands of some Hemiptera, found it was innocuous.

The great damage to infected trees is caused by the fact that the soft, spongy tissue comprising the gall-like swellings gradually hardens and then cracks. These cracks enlarge owing to changing weather conditions, and thus allow the entrance of spores of the canker fungus (*Nectria ditissima*), as pointed out by Blomfield (1905) and Theobald (1909), and observed by the author. The question as to whether the product of the salivary glands exerts the influence on the wounds suggested by Riley and Blomfield is not at present established.

The photographs (Text-figs. A, B, C, and D) reproduced in this paper show parts of a young apple tree taken from a tree

in the laboratory orchard, (i) at the end of first season of infection, (ii) during the second season after infection with *S. lanigera*.

A remarkable feature of aphids is the extraordinary

TEXT-FIG. A.

TEXT-FIG. B.



TEXT-FIG. A.—*Schizoneura lanigera* attacking branch of young apple tree (Cox's orange pippin), the second season after infection. (Photograph taken from experiment trees in the laboratory orchard.)

TEXT-FIG. B.—*Schizoneura lanigera* establishing itself on an injured branch of an apple tree.

numbers of young that may be produced during a season. Reproduction is largely carried on by parthenogenetic females, which give rise to numbers of living young. Throughout successive generations a series of different forms may be produced, thus resulting in a complex polymorphism. These



forms may be winged viviparous females, apterous viviparous females, sexual males and females, with intermediate forms, the larvæ or "lice" and nymphs.

TEXT-FIG. C.



TEXT-FIG. D.



TEXT-FIG. C.—*Schizoneura lanigera* establishing itself in a wound caused by a branch being carelessly broken away from an apple tree.

TEXT-FIG. D.—*Schizoneura lanigera* attacking branch of young apple tree (Cox's orange pippin), three months after infection. (Photograph from experiment tree in the laboratory orchard).

As illustrating the general life-history of aphids and the relation of these different forms in the same species, it would perhaps be well to briefly describe the life-history of *S. lanigera* as far as is known at present.

The "mother queen"<sup>1</sup> may be found throughout the year on infested apple trees in crevices of the bark. It differs somewhat from the apterous viviparous female, in that the body is stouter, and of a shorter oval contour, and the legs and antennæ are shorter. She produces living young or "lice," which collect round her and form a colony.

The members of the colony secrete a number of white, waxy threads from the dermal wax glands, in which they become imbedded. The lice moult in due course and in two or three weeks become apterous viviparous females (closely resembling the queen-mother but smaller), capable of producing living young. This method of reproduction continues throughout the summer. Towards the end of summer some of the lice may develop into nymphs with a large thorax and two pairs of imperfect wings. These nymphs develop into winged viviparous females which may migrate to other apple trees and produce new colonies of living young. Reproduction goes on in this way until late in the autumn, when sexual males and females may be produced. According to Marlatt (1897, p. 3), who cites the observations of Howard and Pergande, these winged viviparous females, which appear about October or November, give rise to a "true sex generation" of lice, the females of which lay a single "winter-egg." The sexual forms of *S. lanigera* are rare. They are apterous, small in size, and much reduced in structure, the mouth parts being atrophied. The oviparous female lays a single egg and then dies. The fertilised eggs are laid near the base of the tree, and remaining in the cracks of the bark throughout the winter, hatch out the following spring, producing larvæ which develop into mother-queens. According to observations made by Theobald (1897), some of the adults migrate into the soil during winter and attack the superficial roots, returning to the aerial portion of the tree in spring.

<sup>1</sup> Buckton (1860) assigned the term "queen aphid" to the immediate issue produced from the egg which becomes the founder of a colony. German authors use the terms "Stammutter" and "Fundatrix."

## IV. GENERAL BODY FORM (Pl. 38, figs. 1, 2, and Pl. 41, fig. 35).

The **body** of the apterous viviparous female is oval in outline. Its **colour** varies from a dull brownish-purple to a richer plum shade, the legs, antennæ, and beak being slightly paler.<sup>1</sup> It presents a well-marked segmentation into head, thorax and abdomen. The head of aphids, according to Huxley (1858, p. 230), consists hypothetically of six segments, but it is usually referred to as the first segment. The thorax consists of three segments, and the abdomen, theoretically, of eleven; but the terminal segments of the body are considerably reduced and only nine abdominal segments are visible. The lateral margins of the abdomen are often well developed, forming a wrinkled border or *connexivum*. In fully fed gravid females however, the body is much distended, the dorsum being strongly convex, and the *connexivum* less pronounced.

Completely covering the body is a flexible, chitinous cuticle or **integument**, which is stouter on the head and coxæ.

The **head** is strongly deflexed under the anterior end of the body, and is produced on its dorsal or anterior face as a pointed upper lip or **labrum** (*lbr.*), which lies above the mouth parts.

On its postero-ventral surface is developed the conspicuous beak or **proboscis** (*pr.*), which in repose, lies on the venter between the coxæ. It has a well-defined longitudinal groove (*l. g.*) on its anterior face, in which lie the delicate, chitinous setæ (*md.*), (*mx.*). These structures, on account of their delicate, hair-like appearance, are generally known as the "setæ."<sup>2</sup> In the following description I shall refer to

<sup>1</sup> When crushed between the fingers, a reddish blood-coloured fluid is squeezed out of the body, and hence this species is known to German authors as the *Blutlaus*. Sorby (1871, p. 351), who made some chemical observations on this colouring matter, calls it "aphidiene."

<sup>2</sup> The term "setæ" means stiff hairs or bristles, and its application to these mandibular and maxillary structures in aphids is misleading. It is however, so extensively used in the literature that I have retained it throughout this paper.

them as the **mandibular setæ** (anterior setæ), and **maxillary setæ** (posterior setæ). They are the "Stechborsten," "Russel-stilette," etc., of German authors.

The **mouth** or oral opening, which is not visible on external examination, is bounded by the oral appendages; and two lateral growths of the wall of the head form a more or less enclosed **buccal cavity** round it.

On the dorsal surface of the head is borne a pair of six-jointed **antennæ** (*a.*), behind which, situated on the sides of the head, are the **eyes** (*oc.*).

Distributed over the dorsal surface of the body are groups of **wax-secreting glands** (*w. g.*), the segmental arrangement of which is clearly indicated by the plate-like, facettèd areas of the integument. These facettèd integumental areas mark the position of groups of large unicellar dermal glands, which secrete the familiar masses of waxy threads. A pair of breathing pores, or **spiracles**, is situated on the ventral surface of the pro- and meta-thoracic segments (*p. s.*), (*m. s.*), but these structures are absent from the meso-thorax. There is also a pair of spiracles on the ventro-lateral surface of each of the first seven abdominal segments (*a. s.*, 1-7).

Two crescentic tubercles (*cn.*) are situated on the dorsal surface of the sixth abdominal segment. These structures represent the **cornicles** or "honey tubes" of other aphids such as **Macrosiphum**, etc.

In addition to the openings of the body described above, there are two others, the **anus** (*an.*), and the **genital orifice** (*g. o.*), situated at the posterior end of the abdomen. The former lies beneath the small, ninth abdominal segment, and ventral to it, being separated by the **anal plate** (*a. p.*), is the genital orifice.

On the ventral surface of the thoracic segments are seated the three pairs of **ambulatory appendages**, the first pair being the smallest, and the third pair the longest.

The **sense organs** consist of a pair of small eyes (*oc.*), and two sensory pits (*s. o.*) situated on the two distal joints of each antenna.

## V. THE EXTERNAL ANATOMY.

**The Integument.**—The integument consists of two layers—an outer, chitinous **cuticle**, and an inner, cellular, hypodermal layer, which produces the cuticle.

The chitinous layer is thin and flexible, permitting free movement of the body segments. It is more stoutly developed on the head and legs. The **hypodermis** consists of a layer of epithelium, in which are scattered small, oval nuclei, but cell boundaries are not clearly defined.

Several small integumental hairs (*h.*) are distributed over the body and legs.

**The Wax-secreting Glands** (figs. 2 and 47).—Distributed over the dorsal integument are numerous circular or oval, faceted areas (*w. g.*) or plaques, the "Wachsdriisenplatten" of German authors. They are arranged in transverse rows near the posterior borders of the thoracic and first seven abdominal segments, four on each segment. In addition, there are two on the eighth abdominal segment, and ten on the epicranial region of the head, the latter being situated on a thickened, quadrangular area of the cuticle, lying between the antennæ.

Each plaque consists of several polygonal areas surrounding a central facette, beneath which are situated large glandular cells. When viewed in vertical section (fig. 47), each is seen to consist of a group of large, glandular cells (*w.c.*), one cell under each polygonal facette. These cells form unicellular, dermal, wax-secreting glands, such as are found in many aphids (*Pemphigus*, *Chermes*, *Lachnus*, etc.), and considered by Claus (1867) to be specially modified hypodermal cells. Each of these glandular cells has a finely reticulate cytoplasm (*cy.*), and a prominent deeply staining nucleus (*n.*) situated at the free end of the cell. At the base of each cell there is an irregular lumen (*lu.*), into which the waxy secretion is poured, being passed to the exterior as waxy filaments through the chitin of the polygonal areas.

The Cornicles.—The **cornicles** (Rückenrohre of German authors, the Saffthöcker of Mordwilko [1895] in Lachnuss), in common with other members of the Pemphiginæ, are greatly reduced in *Schizoneura lanigera*. They consist of a pair of small tubercles situated on the dorsal surface of the sixth abdominal segment, near its anterior border. Each tubercle surrounds a semilunar-shaped opening, which is overhung by a lip-like thickening of the integument, its convex border being directed towards the posterior end of the abdomen.

A section through the body in the region of the cornicles (fig. 46) shows that the integument is thickened to form a lip-like structure over the semilunar-shaped fissure (*c. o.*). Two bands of muscles (*m. cn.*) are attached to the integument near the opening of each cornicle, and passing to the ventral surface of the seventh and eighth abdominal segments, control the valve-like movements of this opening.

Lying in the posterior region of the abdomen is a delicate, wax-sac (*w. s.*), which opens at each side into the cornicles. When a living specimen is examined in normal saline solution, the wax-sac is seen to contain a pale-yellowish, oily fluid, somewhat resembling the fat-globules produced by the fat body-cells. In preserved material, however, the contents of the sac become hard, and form a conspicuous, whitish, refractive mass, which lies in the posterior end of the body. This substance is of a waxy nature, soluble in xylol, but insoluble in alcohol, water, and glycerine. In sections mounted after treatment with xylol, the delicate wall of the wax-sac is seen surrounding the empty cavity of the sac, whose contents have been dissolved by the xylol.

The question as to the function of the cornicles in aphids has occupied the attention of many observers. It would appear from the literature that the term "honey-tubes," which is often applied to these structures, is a misnomer.

According to the resumé of the subject given by Horvath (1904), Réaumur (1737), one of the earlier observers, thought that these structures had an excretory function. Bonnet

(1745), was of the opinion that they excreted a sweet fluid and were concerned with the respiratory and excretory system, this view being adopted by Kyber (1815), Morren (1836) and Kaltenbach (1843). Linné (1758) expressed the opinion that a honey substance was secreted from the cornicles, and this view was fairly generally adopted. Of more recent authors, Witlaczil (1882) considered that sugary substances were excreted from the cornicles. Büsgen (1891), referring to analyses made by Knorr on the secretion produced from the cornicles, showed that it must be regarded as a waxy substance. Mordwilko (1995, p. 363) has demonstrated the waxy nature of the substance underlying the cornicles in *Lachnus viminalis*. Horvath (1904), in his paper, "Sur les cornicles on nectaires des Aphidiens," concludes, as the results of observations made, that the cornicles are secretory canals from wax-producing glands, specially differentiated, the product of which is a waxy fluid, which affords a means of protection against the predaceous larvæ of Coccinellidæ and Chrysopidæ. According to this author the honey substance which attracts ants is produced in clear droplets from the anus. Some American authors (Gillette, 1908), have confirmed Horvath's observations. The author has frequently observed in *S. lanigera* large clear and transparent drops emerging from the anus, which were quite colourless. Buckton (1875, p. 47), has described similar drops in the case of *Schizoneura ulmi*. (See also Mordwilko, 'Biol. Centrbl.,' xxvii, 1907, pp. 212 et. seq.)

The Head and its Appendages (figs. 1, 3, 22-24, 35).—The **head** is borne at the anterior end of the body, and bears the oral appendages which surround the mouth. At its proximal end it broadens out to become continuous with the prothorax. Towards its distal end it becomes narrower, and is strongly deflexed. It is divided by a pseudo-articulation of membranous chitin (*a. j.*) into a broad, proximal portion, the **epicranium**, which bears the small eyes and the antennæ; and an anterior tapering portion (*f.r.*), which includes the **clypeus** and **labrum**. This anterior portion is



composed of stout chitin, and is strongly dome-shaped on its anterior face. It freely articulates with the epicranium by means of thin, flexible chitin (figs. 23, 24), and is continued above the mouth as the pointed **labrum** or upper lip (*lbr.*). It has been termed the "frons" by some American writers (Ashmead, 1889, p. 185), and is the "Vorderkopf" of German authors. Bugnion (1911, p. 649) recognises the homology of this structure in Hemiptera with the clypeus of other insects. A slight transverse ridge occurs at the junction of the clypeus and labrum.

The **appendages of the head** consist of the antennæ and the buccal appendages, the latter comprising the labrum, a pair of mandibles, the maxillæ, and the proboscis or beak (second maxillæ).

The **buccal appendages** of aphids are now generally accepted as homologous with the mouth parts of other insects as follows: the proboscis represents the labium, its anterior wall being continued beneath the mouth as a small underlip<sup>\*</sup> or hypopharynx; the anterior pair of setæ the mandibles; the posterior pair of setæ the maxillæ ii, the labrum being distinct as a triangular prolongation of the clypeus. Smith (1892, p. 189) considers the setæ represent the lacinia and stipes of the maxillæ, developed as in the Diptera. This conception of the buccal appendages of Hemiptera was, however, proved to be erroneous by Marlatt (1895, p. 241), but Smith states in a later paper (1898, p. 176) that he is still of the opinion that the proboscis and setæ are maxillary structures, and that no trace of mandibular structures occur in any present Hemipterous form. Marlatt (1895, pp. 241-249), as a result of careful investigations on the structure of the mouth parts of *Cicada*, shows that the mandibles in this insect are represented by two small sclerites and the anterior setæ, to which the swollen bases of the latter structures are attached. Similarly the maxillæ are represented by two narrower sclerites, to which the posterior setæ are attached. In the lower families of Hemiptera Marlatt states that these sclerites are minute or obsolete.

Smith (1892) observed the presence of these sclerites in Hemiptera, and Marlatt remarks that prior to this author's work the mandibles and maxillæ were supposed to be represented by the setæ alone.

Marlatt (1895, p. 247) states in error that the mandibular setæ in aphids become intimately united; it is the posterior pair of setæ (the maxillæ) which fuse, and not the mandibular setæ.

Bugnion (1911, p. 643), who cites Heymons (1896-8), "Die Mundtheile der Rhynchota," 'Entom. Nachr. Jahrg.,' xxii, No. 11, p. 173), points out that this author showed that the setæ in Hemiptera only represent part of the mandibles and maxillæ, the other portions of these structures being more or less fused with the wall of the head.

I have not observed any chitinous sclerites attached to the setæ and extending from the wall of the head, except those mentioned above as the mandibular and maxillary chitinous rods. These are evidently supporting structures for the setæ. The basal portion of each seta is not in any other way connected with the wall of the head. The insertion of the protractor muscles on the mandibular rods would seem to show that these rods are part of the mandibles.

The proboscis is now generally accepted as being homologous with the labium of other insects.

As regards the function of the buccal appendages, the proboscis acts as a supporting structure for the setæ and is not used as a piercing organ. Marlatt (1895) has made observations verifying this point. The close fusion of the maxillary setæ in the proboscis groove forms a fine canal along which the plant juices are drawn.

The relationship of the buccal appendages will be best understood from the series of sections through the head and anterior part of the body, shown in figs. 6-12, 23-24, 25-31.

The **labrum** is the anterior prolongation of the clypeus. It tapers towards its free end, and is slightly grooved on its posterior or internal face, the chitin of the external face being marked with a few minute, transverse ridges.

The **proboscis** (*pr.*) is formed by an evagination of the integument at the infra-posterior end of the head. It may be withdrawn into the body for some distance, somewhat after the manner of an inverted glove finger, the integument forming a sheath round it, as shown in fig. 34. I have seen specimens with about a half of the beak retracted. It consists of three segments—a long proximal segment, a short middle segment, and a tapering distal segment, at the extremity of which are a few sensory hairs (*s. h.*). A well-defined **longitudinal proboscis groove** (*l. g.*), in which lie the mandibles and maxillæ, runs along the length of its anterior face.

Situated beneath the mouth is the small **hypopharynx** (*h.*) which supports the chitinous salivary pump (*s.*) and is continuous with the labium (proboscis).

The **mandibles** (*md.*) consist of a pair of delicate, chitinous structures which emerge from the head, at the side of the mouth, and extend along the proboscis groove. They are finely pointed at the distal end.

At their proximal ends, which are situated in the head, they are greatly enlarged, and each is attached to a stout **mandibular chitinous rod** (*m. r.*).

The **maxillæ** (*mx.*) consist of two similar structures, being situated immediately posterior to the mandibles, but before leaving the head to enter the buccal cavity, they fuse to form a single seta, which lies between the mandibles in the proboscis groove.

An elongate **maxillary chitinous rod** extends from the wall of the clypeus beneath each maxilla with which they fuse.

At the sides of the mouth the lateral walls of the clypeus are produced downwards as two-lobed structures, which partly enclose the buccal cavity. This is shown in the series of transverse sections through the head (figs. 6–12). It will thus be seen that the labrum, labium, and these lateral lobes of the head form a more or less enclosed **buccal cavity** (*b. c.*) surrounding the mouth.

I shall discuss the oral appendages in greater detail when describing the digestive system.

The **antennæ** (fig. 4) are composed of six articles, of which the third article is conspicuously longer than the others. The two proximal articles are about equal in length, the basal one being slightly broader. The third article is cylindrical in shape, and almost as long as the three distal articles together. The fourth and fifth articles are slightly broader distally, and the terminal one attenuates abruptly at the distal end, forming a short, blunt process, which bears a few sensory hairs. Each of the two distal articles bears a sense organ (*s. o.*), which consists of a circular pit, surrounded by a ring of sensory hairs. A few hairs are scattered over the surface of the articles.

There are no compound eyes such as are found in the winged viviparous stage. Two **small eyes**, each consisting of three **tubercles**, are borne behind the antennæ, one on each side of the head. As seen in section (fig. 49), they consist externally of three transparent convex **tubercles**, beneath which are grouped, in close contact, three densely pigmented areas (*om.*) These areas are somewhat pear-shaped, the long axes being at right angles to the surface of the head, and the tapering portion internal. A delicate strand of nerve-fibres (*oc. n.*) passes from each eye towards the brain. The apterous viviparous female is sluggish in habits, living in the dark cracks of galls or imbedded amongst the members of the colony, which accounts for the poorly developed eyes.

The Thorax and Ambulatory Appendages.—Each of the thoracic segments bears on its dorsal surface four groups of **wax-secreting glands**, and on its ventral surface a pair of legs. Situated on the ventral surface of the prothorax, near its posterior border, are the two **pro-thoracic spiracles** (*p. s.*). Two **meta-thoracic spiracles** (*m. s.*) are situated near the anterior border of the meta-thorax.

The **legs** are composed of five articles—a small basal article or coxa (*cox.*), a short cylindrical trochanter (*tro.*), the femur (*fe.*), tibia (*ti.*) and tarsus (*ta.*).

The **femur** is elongate and slightly broader distally. The **tibia** is elongate and of uniform thickness. The distal article

or **tarsus** consists of a small basal joint (*b. ta.*) and a longer, slightly curved, distal joint, which bears a pair of curved claws (*cl.*) and a few tactile hairs.

The anterior pair of legs is the shortest, the mesothoracic legs being slightly longer, and the third pair the longest. A few hairs are borne on each of the articles.

The **Abdomen**.—The **abdomen** consists of nine visible segments.<sup>1</sup> The terminal or ninth segment (*abd. ix*) is very small, and is not prolonged into a cauda or tail, such as is found in many aphids. Situated below the terminal segment, opening on the dorsal surface of the body, is the **anus** (*an.*), immediately beneath which is the conspicuous **anal plate** (*a. p.*). This latter structure is an arched, lobe-like development of the integument, which extends beneath the anus and the genital orifice, and bears numerous stout hairs.

The transverse **genital orifice** (*g. o.*) is situated ventral to the anus, being overhung by the anal plate. On the ventral border of this opening the integument is developed to form a small semilunar-shaped **genital plate** (*g. p.*), which bears stout hairs.

The **abdominal spiracles** are situated on the ventral surface, near the lateral margins of the body, in the wrinkled membranous chitin at the anterior borders of the first seven segments.

<sup>1</sup> The number of segments in the abdomen of aphids is a disputed point, owing to the modification of the terminal segments. Balbiani (1869, p. 64) refers to the work of Lacaze-Duthiers (1853), 'Recherches sur l'armure genitale femelle des Insectes,' who considers that the typical number in insects is eleven. According to Buckton (1875, p. 20), this latter author has shown that in most Hemiptera there are three segments intervening between the genital orifice and the anus, and as Balbiani observes, the absence of the terminal segment in aphids must be attributed to the atrophy of one of the post-genital segments. Kaltenbach (1843) considers there are only nine segments. Tullgren (1909) considers there are only nine visible in Schizoneura, and I have adopted his terminology.

## VI. THE INTERNAL ANATOMY.

The internal anatomy of *Schizoneura lanigera*, so far as I am aware, has not been studied in any detail before. Indeed, considering the great economic importance of the Aphididæ, our knowledge of the anatomy of this family is very small. Several of the earlier workers on aphids, such as Dufour (1833), Morren (1836), Kaltenbach (1843) and Buckton (1875-82), treated in a general way of the anatomy of the group. Later, Balbiani (1866, etc.), Mark (1877), Witlaczil (1882, 1884), and Will (1888), have done much to further our knowledge of the internal structure and histology of these important insects. Of more recent contributions to the literature of aphid anatomy, the works of Dreyfus (1889 and 1884), Krassiltschik (1892-3) on *Phylloxera vastatrix*, Mordwilko (1895) on the anatomy of *Trama* and Lachnus, Flögel (1904) on *Aphis ribis* and Grove (1909) on *Siphonophora rosarum* may be cited.

## Distribution of the Internal Organs.

When the dorsal integument is carefully removed under normal salt solution from a living, apterous viviparous female of *S. lanigera*, as shown in fig. 5, numerous olive-coloured **fat body cells** are seen, lying beneath which are a number of oval yellowish-brown **embryos** (*e.*), in various stages of development. These embryos are contained in long, thin-walled tubes or the **ovarian cæca** (*p. c.*), which are transversely constricted along their length to form several large **ovarian chambers**, in which the embryos are borne. The cæca are arranged in two groups, one group on each side of the median line, with about four or five cæca in each, and extend throughout the greater part of the body. In the posterior region of the abdomen the tubes from each side lead into a wide **oviduct** (*od.*), and the two oviducts enter into a median, muscular chamber, the **vagina** (*v.*), which leads to the **genital orifice**.



On removing the embryos from the dorsal surface the whitish-yellow coils of the digestive canal are seen lying beneath.

The **alimentary canal**, as seen in side view (fig. 35), leads from the **mouth** into a well-defined **pharynx** (*ph.*), which structure passes upwards through the head, and leads into the narrow, tubular **œsophagus** (*œs.*). The **œsophagus** passes in the median line over the thoracic ganglion (*t. g.*), and enters into the stomach (*st.*) or sac-like dilation of the mid-gut. The **stomach** narrows towards its posterior end, and leads into the coiled **intestine** (*i.*), from which the **rectum** (*r.*) passes in the median line over the vagina (*v.*) to the **anus** (*an.*).

The **salivary glands** lie obliquely in the thorax, above the **œsophagus**, and consist of a small anterior gland (*s. a.*) and a large posterior gland (*s. p.*), situated on each side of the median line. The salivary duct from each side passes beneath the thoracic ganglia, and both unite in the mid-ventral line to form a median salivary duct (*s. d.*) which leads to the **salivary pump** (*s.*), situated at the posterior end of the buccal cavity.

The **supra-œsophageal ganglia** occupy the greater portion of the head. Extending beneath the **œsophagus** is an elongate ganglionic mass, which comprises the sub-œsophageal ganglion, with its two broad commissures, and the fused thoracic and abdominal ganglia.

The **respiratory organs** consist of fine tracheal tubes, which ramify over the body in two definite systems, a dorsal and a ventral tracheal system, the main tracheæ from which lead on each side of the body to the nine pairs of spiracles.

Four conspicuous bands of **longitudinal muscles** extend along the floor of the body, two on each side of the median line, and four similar bands extend beneath the dorsal segment.

#### A. The Digestive System (Pl. 38, fig. 5; Pl. 41, fig. 35).

The **mouth** or oral opening is situated on the ventral surface of the head, being bounded anteriorly by the labrum (*lbr.*),



and posteriorly by the hypopharynx (*h.*) and proboscis (*pr.*). Laterally it is bounded by the lateral lobes of the clypeus, mandibles (*md.*), and the maxillæ (*mx.*), and leads into the suction canal formed by the fusion of the maxillæ.

**The Pharynx.**—The mouth leads upwards into the **pharynx**. This structure is a distensible chamber lined with chitin, which is continuous with the cuticle of the body. Its posterior or ventral wall is strengthened by a stout layer of chitin, but its anterior wall is composed of flexible membranous chitin. As seen in transverse sections (figs. 12–20) the pharynx is crescentic in shape, its greatest transverse diameter being near the oral end. Its walls in this region are thickened to form two hollow, dome-shaped protuberances (*p. p.*) or ridges, the “*Naröiden*” of Dreyfus (1894) in *Phylloxera*. The lumen of the pharynx in this region is very narrow, and divaricator muscles (*m. ph.*) pass from its dorsal wall to the wall of the clypeus. Two folds of chitin extend from the clypeus to the wall of the pharynx protuberances, with which they fuse.

The pharynx is well supplied with muscles. Attached to its anterior flexible wall are several bands of divaricator muscles, which extend to the anterior wall of the clypeus. These muscles are divided into fine tendons at their attachment on the chitinous walls of the clypeus. By means of these muscles, the anterior wall of the pharynx is drawn outwards and the lumen of that structure is greatly enlarged, the plant juices being drawn into it through the mouth. I have pointed out that the lumen in the region of the **pharynx protuberances** is very narrow, and ventrally has stout chitinous walls. When the divaricator muscles of this region are relaxed, the entrance into the pharynx chamber is almost closed, preventing the plant juices from returning into the mouth. As the other divaricator muscles of the pharynx are relaxed, the anterior wall of that structure, on account of its flexibility, tends to regain its original shape, and the cavity, being thus greatly reduced in size, the juices are forced backwards into the **œsophagus**.

The Endoskeleton of the Head (Pl. 38, fig. 3; Pl. 39, figs. 6-24; Pl. 40, figs. 25-32).—The **chitinous endoskeleton** of the head consists of an arrangement of chitinous rods or endosternites, which, in addition to giving support to the head, afford attachment for muscles of the head and its appendages. The endoskeleton in *S. lanigera* agrees closely with that described by Dreyfus (1894) in *Phylloxera* and Mordwilko (1895) in *Lachnus*.

Situated in the posterior portion of the head, beneath the supra-œsophageal ganglion, is a hollow, chitinous plate or bar (*t.*), which forms the central support of the endoskeleton. This structure corresponds to the "Chitinstab" of Mordwilko (1895) in *Lachnus*, named after Witlaczil (1882). It is also the "arcus superior" of Mark (1877), Krassiltschik (1892-3), and other authors. Grove (1909) suggested the term "**transverse bar**" for the corresponding structure in *Siphonophora rosarum*, and throughout my description of *S. lanigera* I shall use this term.

Four hollow rods of chitin are fused with the transverse bar and pass from it to the walls of the head. They are as follows:

A pair of **antero-dorsal rods** (*a. d.*) (the *Arcus inferiores*) of Mark (1877), Dreyfus (1894) and Mordwilko (1895), pass dorsally, one from each end of the transverse bar in an antero-lateral direction to the roof of the head, joining it at the junction of the clypeus and epicranium. From here a ridge of chitin (*costæ inferiores* of Mark, Dreyfus, etc.) passes on each side of the clypeus along its lateral walls, with which they fuse.

A pair of **antero-ventral rods** (*a. v.*) embracing the *arcus superiores* and *costæ superiores* of Dreyfus and Mordwilko pass ventrally from the ends of the transverse bar, in an antero-lateral direction, towards the infra-posterior border of the head, each being then reflected along the lateral walls of the clypeus to a position opposite the swollen proximal ends of the mandibles, where they fuse with the wall of the head. From this region, on each side, a stout chitinous

rod passes at right angles into the head towards the proximal end of each mandibular seta. A narrower and more elongate rod passes beneath each maxillary seta. These structures I have called the mandibular (*m. r.*) and maxillary (*mx. r.*) chitinous rods.

The **mandibular rods** are stouter than the maxillary rods, and expanding at the base, fuse with the continuation of the antero-ventral rods and the antero-lateral walls of the clypeus.

The **maxillary rods** are attached at the base of the mandibular rods, from whence they curve ventrally, pass beneath the proximal portion of the maxillary setæ and fuse with them.

Two thin rods of chitin (*v. r.*) ("Chitinfortsatzestabchen" of Mordwilko), to which are attached a few muscles from the salivary pump, arise from the posterior end of the buccal cavity and extend beneath the pharynx towards the transverse bar where they fuse.

**The Buccal Appendages.**—The **buccal appendages** consist of the **labrum**, **mandibles**, first pair of **maxillæ**, and the proboscis or **labium**.

The labrum and labium have been described above under the head appendages.

The **mandibular setæ** (*m. r.*) are situated in the head below the pharynx, being supported by the two mandibular chitinous rods described above. At their proximal ends they are greatly enlarged, but as they pass towards the buccal cavity they become considerably attenuated and approximate closely to one another in the median line. They are finely pointed at their distal ends, and each is composed of stout chitin with an extremely fine cavity running throughout its length. After leaving the head they pass through the buccal cavity and extend along the proboscis groove. The flexible chitin of the buccal cavity is attached to the walls of the mandibles, which thus permits of free movement of these structures (fig. 33). When they are retracted into the head, the sheath is drawn inwards after the manner of an inverted finger of a glove.

The **maxillary setæ** (*mx. r.*) are situated immediately posterior to the mandibles, which they resemble in structure.

As they enter the buccal cavity they fuse to form a single chitinous seta, which extends between the mandibles along the proboscis groove and encloses two minute canals.

Inserted on the internal face, at the proximal end of each of the mandibular setæ, is a strong **mandibular retractor muscle** (*m'. m.*), both of which extend in a postero-dorsal direction to become attached respectively to the right and left antero-dorsal rods of the endoskeleton, near the ends of the transverse bar. A similar **maxillary retractor muscle** is attached to each of the maxillary setæ (*m'. mx.*), and extend to the post-ventral rods, being attached to them near the ends of the transverse bar.

**The Retort-shaped Organs.**—Extending from the proximal end of each of the setæ is an elongate, compact mass of small cells possessing well-stained nuclei. They are shown in fig. 22 (*md. o.*), (*mx. o.*). These structures are related to the peculiar, **retort-shaped organs** (*re. o.*) found lying in the thorax and posterior part of the head in well-developed embryos of *S. lanigera* and other aphids. In advanced embryonic stages they are large and conspicuous, being of a characteristic retort shape. From the neck of each retort a long, fine, chitinous tube, the so-called "seta," is produced, which may frequently be seen coiled round these organs in the anterior part of the embryo.

These retort-shaped structures are bounded externally by a chitinous membrane and an epithelial layer of cells, with elongate, flattened nuclei; and the interior of the retort is packed with a mass of small cells possessing deeply staining nuclei. As development proceeds from the larval stage to the adult they appear to degenerate, so that in the adult apterous viviparous female, after completion of the moultings, they are sometimes difficult to make out. Krassiltschik (1893, p. 9) says that in fully developed adults of *Phylloxera vastatrix* the retorts completely disappear. I have observed them in all the longitudinal sections of the apterous viviparous females of *S. lanigera* examined, although they usually appeared to be in a degenerate condition.

Mecznikow (1866, p. 462) states that during the development of Aphididæ, the structure in the early embryonic stages corresponding with rudimentary mandibles and maxillæ disappear, and that the retort-shaped organs found in more advanced embryos become elongated at the neck end and produce the "Russelstilette," and Mayer (1875, p. 335), in describing the origin of the "Stechborsten" in *Pyrrochoris apterns*, states that the retort-shaped organs observed throughout the development of this insect, are to be considered as the "Bildungstatten" of those structures. Witlaczil (1882, p. 415) is of the opinion, that the embryonic "Anlagen" of the mandibles and maxillæ do not disappear, but sink into the head, and give rise to the retort-shaped organs.

I propose to discuss the question as to the structure of these organs and their relation to the mandibles and maxillæ in greater detail when dealing with the anatomy of the larva.

**The Salivary Glands.**—The **salivary glands** are whitish glandular bodies, situated in the prothorax and posterior region of the head. They consist of two pairs<sup>1</sup> of glands, which lie above the thoracic ganglion, one pair on each side of the œsophagus. Each pair comprises a large posterior gland and a smaller anterior gland.

The **posterior salivary gland** (*s. p.*) is large, oval in shape, and lies obliquely in the prothorax.

The **anterior salivary gland** (*s. a.*) is small, spherical in outline, and situated close to the anterior end of the posterior gland.

A narrow duct leads from the anterior end of each posterior gland, near to which it receives a small duct from the anterior gland. The **salivary duct** from each side passes

<sup>1</sup> In some Aphids three glandular bodies have been described, as in *Phylloxera vastatrix* (Krass, 1893); also in *Aphis ribis* (Flögel, 1905). In these cases, as Mordwilko (1895) points out, the large posterior gland described above, may be bi-lobed. The small anterior gland, on account of its small size, was overlooked by some of the earlier workers. Mark (1877) has figured the salivary glands of *S. ulmi*, which correspond closely to those in *S. lanigera*.

in an antero-ventral direction beneath the thoracic ganglion, and both unite in the mid-ventral line to form a bulbous expansion in the infra-posterior region of the head. From here the salivary duct (*s. d.*) continues beneath the large pump muscle (*m.*) as a single median duct, and decreasing considerably in size, leads into the **salivary pump** (*s.*), which is situated in the hypopharynx at the posterior end of the buccal cavity.

The salivary glands are simple in structure (fig. 41). The posterior gland is a sac-like body consisting of an epithelial layer of large, conical cells, which bulge into the irregular cavity (*lu.*) of the sac. These cells possess fine, granular cytoplasm (*cy.*) and prominent, deeply staining nuclei, and border on the irregular lumen of the gland, which leads at the proximal end, by a narrow channel, into the salivary duct. The cells in the posterior portion of the gland appear to be filled with secretion, the nuclei are larger, and the cell contents appear less granular than in the anterior portion.

The cell boundaries of the anterior glands are not well defined, and the lumen is almost entirely reduced. A few large, deeply staining nuclei are present, imbedded in a granular cytoplasm.

The walls of the salivary duct are comparatively thick, and enclose an extremely fine lumen, along which the salivary secretion is passed. Several nuclei are seen in the walls, but cell boundaries are not well defined.

The Salivary Pump.—Lying beneath the anterior end of the pharynx, being supported by the small hypopharynx, is the **salivary pump** (*s.*) or “Speichelpumpe” of German authors, into which the salivary duct conveys the products of the salivary glands, which then pass along a fine canal in the fused maxillæ. This structure corresponds in position to the “Wanzenspritze” described by Wedde (1885) in the Rhynchota. It has been observed by Mayer (1873) in *Pyrrhocoris*, Mark (1877) in *Chionaspis*, Witlaczil (1882) in Aphids and Coccidæ, Dreyfus (1894) in *Phylloxera*, and other authors. Krassiltschik (1892–93)

has described its structure in *Phylloxera vastatrix*. Kershaw (1911) has recently described the structure of the pump in *Pristhesancus papuensis*, a species in which this organ is well developed. My observations on the salivary pump agree closely with those of Dreyfus (1894) in *Phylloxera vastatrix*.

When seen in longitudinal section (fig. 24), the salivary pump in *Schizoneura lanigera* consists of a **salivary chamber** (*s.c.*) or "Kolben," whose outer walls form the **cylinder** (*cd.*) and a strong, distal portion or shaft, which is strongly chitinised and laterally compressed, its chitinous walls being continuous with that of the hypopharynx.

At its closed proximal end, as will be seen in vertical sections through the head (figs. 31, 33), the cylinder is greatly enlarged, its outer walls being strongly chitinised. Its posterior wall is membranous, and invaginated into the cylinder, thus forming a space, the salivary chamber, between this wall and the outer walls of the cylinder. On this posterior concave wall is inserted the large **pump muscle** (*m.*), which extending beneath the pharynx, becomes attached to the transverse bar of the endoskeleton.

The salivary duct leads beneath the pump, and apparently enters into the salivary chamber on its ventral surface, but I have not been able to definitely trace its entry into the chamber. Kershaw (1911) describes and figures in *Pristhesancus papuensis* a valvular entrance from the salivary duct into the cylinder, or what this author terms the "syringe barrel," but I have not observed this in *Schizoneura lanigera*.

The working of the pump is largely controlled by the large pump muscle (*m.*). When this muscle contracts, the invaginated posterior wall of the cylinder is pulled outwards, and the cavity of the salivary chamber being enlarged, the contents of the salivary ducts are drawn into it. When the muscle is relaxed, the posterior wall of the cylinder, which is membranous and elastic, tends to regain its original position, and the lumen of the cavity being thus reduced, the saliva is



forced along the pump shaft into a fine canal down the fused maxillæ.

The **Œsophagus**.—The **œsophagus** (*œs.*) leads as a narrow, straight tube from the posterior end of the pharynx, and passing through the circum-œsophageal ring, over the transverse bar, extends in the median line above the thoracic ganglia.

In the mesothorax it enters into the stomach or dilated region of the mid-gut.<sup>1</sup> When examined as a fresh preparation in normal salt solution, it appears as a whitish-grey, semi-transparent tube, with comparatively thick walls, which surround a very small lumen.

The **walls of the œsophagus** consist of an epithelial layer composed of granular protoplasm, in which are scattered small, elongate nuclei, but cell boundaries are not well defined. A thin, outer coat of connective tissue surrounds the epithelium.

The **mid-gut**.—The **mid-gut** extends somewhat obliquely in the median line, its anterior portion being considerably enlarged to form the stomach, which is the widest part of the alimentary canal. As will be seen in longitudinal sections, the œsophagus is invaginated for some distance into the stomach, its walls being reflected back to become continuous with the walls of that structure, thus forming an effective **œsophageal**

<sup>1</sup> This expanded portion of the digestive tract in aphids is sometimes referred to as the "crop." The crop in insects is associated with the stomodæum, being derived from ectoderm, and lined with a chitinous intima. The cells composing the wall of the expanded portion of the mid-gut in *S. lanigera* agree in character with those in other parts of the wall of the mid-gut. Further, the place where the œsophagus terminates and the large cells of the expanded portion of the gut commence is clearly defined. There is no chitinous intima in this region. Of course, the absence of a chitinous intima is not in itself proof of its mesodermal origin, as a chitinous lining is found in the mid-gut of many insects, as shown by Schneider, but in these cases its origin is doubtful. This structure must be considered as part of the mid-gut, and the term "crop" is erroneous. I shall refer to this portion of the gut embracing the œsophageal valve as the stomach. It is the "Magen" of Dreyfus (1894) and others.

**valve** (fig. 48), which prevents the regurgitation of plant juices from the mid-gut to the œsophagus.

A band of **connective tissue** (*c. t.*) extends from the œsophagus across the shoulder of the œsophageal valve, forming a kind of ligament which is continuous with the connective-tissue coat of the œsophagus and stomach.

The **stomach** forms a thick-walled sac with a wide lumen, which is usually filled with plant juices. Its wall consists of an epithelial layer of large, conical cells, the free ends of which bulge into the cavity of the sac (fig. 40). Externally there is a thin layer of connective-tissue. Towards the posterior end of the stomach the lumen decreases in size, and the cells become flatter.

At the base of each cell is a prominent deeply-staining nucleus, which is embedded in a mass of granular secretion. The granules are also densely crowded throughout the cytoplasm. It is probable that these cells function as digestive glands, and pour digestive secretions into the lumen of the stomach. Along the free end of the cells there is a well-defined margin, which, bordering the lumen, stains deeply with eosin.

At its posterior end the stomach narrows considerably, and becomes continuous with the **coiled intestine**. The structure of the walls of the latter closely resembles that of the stomach, but the cells are not so conical, and the granular elements in the cytoplasm not so dense. The lumen of the intestine is greatly reduced, and usually tri-radiate or irregular in section.

When seen in fresh preparations the mid-gut is whitish-grey in colour, semi-transparent, with the large nuclei of the epithelial cells showing conspicuously through the walls. The general course taken by the intestine may be described as follows (figs. 5 and 35).

The stomach extends obliquely in the median line to about the third or fourth abdominal segment, where it leads into the intestine (*i.*), which turns abruptly on itself in an antero-ventral direction. In the posterior half of the thorax the

intestine increases in size, and lies transversely across the median line above the anterior end of the stomach, turning abruptly on the left of the median line in a posterior direction beneath that structure. About the third abdominal segment it narrows considerably, and passes beneath the stomach towards the thorax, where it again bends at right angles across the median line, and leads into the rectum (*r.*), which continues in a postero-median direction towards the anus.

The coils of the alimentary canal lie beneath the large ovarian cæca, near the ventral surface of the body, and are held in position by strands of connective tissue and fine ramifying tracheæ.

The Rectum.—The **rectum** or posterior chamber of the hind-gut continues in a post-median direction as a thin-walled tube, and passing between the two oviducts, extends upwards above the vagina to the anus. In some individuals it is distended in a sac-like manner, but attenuates towards the anus.

The structure of the wall of the rectum (fig. 37) differs from that of the mid-gut. It is composed of an irregular epithelium which bounds the lumen. The cell walls are not clearly defined, but conspicuous nuclei are embedded throughout the epithelium. A delicate, chitinous intima, continuous with the body integument, extends for some distance along the rectum.

Two bands of divaricator muscles are attached to the rectum near the anus, and pass to the body-wall.

Malpighian tubes are absent in *S. lanigera*. Kowalevsky (1889), as cited by Mordwilko (1895, p. 353) and other authors, has shown that in insects which have no Malpighian tubules, the cells of the rectum wall may have an excretory function.

#### B. The Nervous System (figs. 35 and 36).

The **nervous system** is concentrated in the head and thorax. It consists of a pair of fused **supra-œsophageal ganglia** (*s. g.*),

which occupy the greater part of the head; a pair of fused **sub-œsophageal ganglia**; and an elongate **median ganglion**, lying in the thorax beneath the œsophagus. The latter represents the fused elements of the ventral nerve chain, and comprises the three fused **thoracic ganglia**, to which the sub-œsophageal ganglia are attached by two broad commissures, and a **median abdominal ganglion**. Nerves are given off from the ganglia to the appendages and other parts of the body.

The **supra-œsophageal ganglia** occupy the epicranial region of the head. They consist of two fused, pear-shaped ganglia, which have their widest part towards the anterior or dorsal wall of the head (figs. 18, 21). Histologically they consist of an outer coat of ganglionic cells with deeply staining nuclei, and an inner, lightly stained area composed of an interlacing mass of nerve fibres.

Near the infra-posterior end of these ganglia, two short **circum-œsophageal commissures** (*cs.*) lead round the œsophagus, and connect with the sub-œsophageal ganglion, forming a loop through which the œsophagus passes.

From the antero-dorsal end of the supra-œsophageal ganglia a pair of antennal nerves (*n. a.* fig. 21) are given off laterally to the antennæ, behind which are a pair of small nerves which pass to the eyes.

The **sub-œsophageal ganglion** (*sb. g.*) is smaller, and is connected with the thoracic ganglia by means of two broad commissures. From its anterior end a pair of small nerves arise, which innervate the proboscis, and another pair innervate the maxillæ and mandibles.

The fused **thoracic ganglia**, together with the **median abdominal ganglion** (*a. g.*), form an ovoid, elongate mass, which extends in the median line along the ventral surface of the thorax.

The histological structure of these ganglia is the same as before, consisting of an outer coat of ganglionic cells possessing nuclei of varying sizes, and an inner, whitish-grey, medullary region, composed of an interlacing network of nerve-fibres.

From each of the thoracic ganglia a pair of nerves is given off laterally, which pass into the legs (*n. l<sub>1</sub>.-n. l<sub>3</sub>.*). These nerves bifurcate before entering the coxæ. From the posterior end of the abdominal ganglion a stout **post-abdominal nerve** (*p.*) extends in the median line beneath the alimentary canal, almost to the posterior end of the body. At its extremity, beneath the vagina, it expands into an irregular club-shaped **post-abdominal ganglion** (*p. g.*), from which nerves pass to innervate the vagina and muscles of the terminal abdominal segments. Several nerves are given off along the length of the post-abdominal nerve. They arise more or less irregularly in pairs, and pass in a post-lateral direction to the abdominal segments, especially innervating the longitudinal bands of muscles on the floor of the body.

c. The Respiratory System (Pl. 38, fig. 5; Pl. 41, fig. 39).

The **respiratory system** consists of a dorsal and ventral system of tracheal tubes, the main trunks from which communicate with the exterior by means of nine pairs of spiracles. Each spiracle consists of a small opening surrounded by a chitinous ring. Each of the first seven abdominal segments bears a pair of spiracles, which are situated on the ventral surface, near the lateral margins of the body.

The two **pro-thoracic spiracles** are situated external to the coxæ on the ventral surface of the prothorax, near its posterior margin. Two similar **meta-thoracic spiracles** (*m. s.*) are situated near the anterior border of the metathorax. There are no spiracles on the mesothorax.

**Dorsal Tracheal System.**—Leading from each spiracle is a short tracheal trunk (*s. r.*), which bifurcates into a dorsal (*d. b.*) and a ventral (*v. b.*) tracheal branch. The dorsal branches from the spiracles pass upwards, more or less parallel to one another, along the lateral walls of the body to the dorsal surface, and then inwards towards the dorso-median line. At some distance from the median

line the dorsal branches from all except the prothoracic spiracles bifurcate, and join to form on each side of it a **main dorsal longitudinal trachea** (*d. t.*), which extends beneath the dorsal integument in a zig-zag longitudinal course, from the prothoracic to the seventh abdominal spiracle.

One of the divisions of the dorsal branch from each of the seventh abdominal spiracles passes inwards, and both fuse in the median line, thus connecting the two lateral divisions of the dorsal tracheal system.

Several small bunches of tracheæ, which ramify over the reproductive organs and alimentary canal and aerate the dorsal longitudinal muscles, are given off from the dorsal longitudinal trachea.

From the prothoracic spiracles a stout trachea (*a. t.*) passes anteriorly, and branching considerably, aerates the cephalic ganglia and muscles of the head, a branch passing to the antennæ.

**Ventral Tracheal System.**—The ventral tracheal branches from the abdominal spiracles pass inwards, more or less parallel to one another, along the ventral surface of the body, and with the exception of the branch from the first and last of these spiracles, bifurcate to form a zig-zag **ventral longitudinal trachea** (*v. l.*) on each side of the median line as in the dorsal system, but in the former case this stout trachea only extends from the first to the last abdominal spiracles.

The ventral tracheal branch from the seventh abdominal spiracle does not bifurcate, the two lateral divisions of the ventral system not being connected at the posterior end, as is the case in the dorsal system.

Several small tracheæ are given off from the ventral longitudinal trachea of each side, and pass inwards to aerate the internal organs and ventral bands of longitudinal muscles.

A small trachea arises from the ventral branch near each spiracle.



The ventral branch from each of the prothoracic spiracles passes towards the median line, and both fuse to form a continuous, stout, transverse trachea (*t. t.*), which extends across the median line joining the two spiracles. Several smaller tracheal tubes arise from it, and aerate the muscles in the head and prothorax. A branch also passes into the first pair of legs.

The prothoracic spiracle is connected with the metathoracic spiracle by a small trachea (*c. tr.*), which arises near the former spiracle, and passing posteriorly, bifurcates, one branch passing into the second pair of legs, and the other joining the second tracheal trunk near the metathoracic spiracle.

The ventral tracheal branch from each of the metathoracic spiracles also fuse in the median line, and form a stout trachea, which lies transversely across the thorax, connecting the two spiracles, and giving off a number of smaller tracheæ, which aerate the muscles of the thorax. A small trachea arises from the tracheal trunk of this spiracle, and passes to the tracheal trunk of the first abdominal spiracle, giving off a branch to the third leg. A branch also passes forward into each of the second pair of legs.

#### D. The Circulatory System.

A tubular **heart** or **dorsal vessel** has been described by Witlaczil (1882, p. 35) as occurring in *Aphis*, and this author described its development in his later work (1884, p. 652).<sup>1</sup> Mordwilko (1895, p. 356) also refers to the presence of a chambered dorsal vessel in *Trama*. Dreyfus (1894, p. 238), on the other hand, was unable to find any trace of this structure in *Phylloxera*; and Grove (1909, p. 26) failed to find it in *Siphonophora rosarum*. I have been

<sup>1</sup> Ich fand bei *Aphis* dass das Herz und die Aorta aus einem Strang von Mesodermzellen gebildet werden, welcher Anfangs solid, durch Theilungseine zellen vermehrt und indem er sich aushölet, wahrscheinlich die Blutkörperchen entstehen lässt. Die venösen Ostien fand ich hier an der Grenze je zweier Segmente des Abdomens.



unable to establish its presence in the apterous viviparous female of *S. lanigera*.

E. The Reproductive System (Pl. 38, fig. 5; Pl. 41, fig. 35; Pl. 42, figs. 45, 46).

The **reproductive organs** of the apterous viviparous female occupy a considerable portion of the body. In general morphology they are simple in structure, and resemble in a general way those described by earlier investigators, such as Dufour (1833), Witlaczil (1882), etc., in other aphids.

They consist of a number of thin-walled tubes or large **ovarian cæca**, a pair of **oviducts**, and a stout, muscular **vagina** which leads to the **genital orifice**, the whole forming the **ovarium**.

The **ovarian cæca** (*p. c.*) are transversely constricted at intervals along their length, forming a series of several **ovarian chambers** (*p. a.*), in which the embryos are developed. The cæca are developed in two lateral groups of about five tubes in each group, and extend over the alimentary tract, throughout the body. In the posterior region of the abdomen they open into the two **oviducts** (*od.*), which pass beneath the rectum and unite in the region of the sixth abdominal segment to form the stout, muscular **vagina** (*v.*), which extends beneath the rectum to the **genital orifice** (*g. o.*).

The large ovarian chambers contain **embryos** in varying stages of development, those in the chambers nearer the genital orifice being more fully developed. In the earlier stages of development these embryos were called "pseudova." The development of the pseudovum has been described by Huxley (1858, p. 215) in an agamic stage of a species of *Aphis*. According to this author, it would appear that the embryos are developed from pseudova, which, surrounded by a vitelline substance, are set free in the chambers of the ovarian cæca, and eventually become changed into cellular germs, from which the germ-layers of the embryos are developed.

The terminal chambers of the cæca are small, and from the distal one is produced a fine thread-like **ovarian ligament**.

The **oviducts** are slightly flattened dorso-ventrally, and have stout muscular walls, with an inner epithelium of irregular, columnar cells bounding the duct.

The **vagina** is flattened dorso-ventrally, appearing narrow in longitudinal sections, but broad when viewed from the dorsal or ventral aspect. As seen in fresh preparations, it is whitish in colour, semi-transparent, and possesses stout, muscular walls.

The walls consist of a thick, outer layer of circular muscles, and a few scattered, inner, longitudinal muscles, with an epithelial layer of irregular cells, bordering the cavity (fig. 42).

As the vagina leaves the genital orifice it bends upwards, the muscular walls being thrown into folds.

The **genital orifice** is wide transversely, and is bounded by the genital and anal plates, which bear several stout bristly hairs.

The **embryos** are squeezed out through the orifice, posterior end first, by the muscular action of the vagina walls. As they emerge from the orifice they are smooth and glistening, the appendages adhering firmly to the sides of the body. The embryos are held by the head in the orifice for a few minutes after birth, and the anterior appendages are first set free from the sides of the body, the other legs being released soon afterwards.

#### F. The Muscular System.

The muscular system is well developed. For purposes of description the distribution of the various muscles may be conveniently treated as follows:

(1) The longitudinal body muscles. (2) The musculature of the head and its appendages. (3) The musculature of the thorax and ambulatory appendages. (4) The dorso-ventral muscles.

(1) The Longitudinal Body-muscles (Pl. 38, fig. 5;

Pl. 41, fig. 39).—These consist of four conspicuous bands of muscles lying along the ventral surface of the body, two on each side of the median line, and four beneath the dorsal integument. They are divided into two, three, or four bundles or fasciculi in each segment.

On the ventral surface the two **internal, ventral longitudinal bands** (*m.v'*), lie one on each side of the median line, and extend from the antero-ventral border of the prothorax to the eighth abdominal segment.

The two **external, ventral bands** (*m.v.*) extend parallel to the lateral margins of the body. They reach from the antero-ventral border of the first abdominal segment to the eighth segment.

On the dorsal surface the two **internal, dorsal longitudinal bands** (*m.d'*) extend from the eighth abdominal segment to the antero-dorsal border of the mesothorax, from which latter position each sends two diverging bands of muscles to the antero-dorsal border of the prothorax.

The **external, dorsal longitudinal muscles** (*m.d.*) extend from the antero-dorsal border of the metathorax to the eighth abdominal segment.

(2) The Muscles of the Head and its Appendages (Pls. 39 and 40).—(A) The **divaricator muscles of the pharynx** (*m.ph.*) are attached along the dorsal or anterior wall of the pharynx, and extend to the anterior wall of the clypeus. From the region of the pharynx protuberances, stout bands of lateral clypeal muscles (*m.p.*) pass outwards to become attached to the side walls of the clypeus.

(B) A few bands of **lateral muscles** (*m.f.*) extend across the lobes of the clypeus.

(C) Lying beneath the pharynx is the conspicuous **pump muscle** (*l.p.m.*), which is attached to the middle of the transverse bar and passes forwards beneath the pharynx, being inserted on the concave wall of the cylinder portion of the salivary pump (fig. 33). This muscle is divided longitudinally, and is spindle-shaped, being wider about the middle of its length.

Two small bands of muscles (*p. m.*) extend from the salivary pump to the ventral rods of chitin (*v. r.*).

(D) Two bands of **elevator muscles of the transverse bar** (*m. b'*) extend from that structure in an antero-dorsal direction, to become inserted on the epicranial region of the head, and two similar bands of **depressor muscles** (*m. b.*) pass from this bar to the post-dorsal border of the head.

(E) Two bands of **depressor muscles of the head** (*m. h.*) are attached at its infra-posterior angles, and pass to the dorsal walls of the prothorax.

(F) The movements of the antennæ are controlled by a band of elevator (*m. a<sup>1</sup>.*) and depressor (*m. a.*) muscles, which are attached to the antero-dorsal rods of chitin, near the ends of the transverse bar, and pass outwards to become inserted on the walls of the basal article of the antennæ.

(G) Two **elevator** and two **depressor muscles of the proboscis** (*m. pr.*) are inserted at the base of that structure and pass by the side of the thoracic ganglia to the dorsal wall of the thorax.

(H) The **retractor muscles of the mandibles** (*m'. m.*) are inserted on the internal face of the proximal ends of the mandibles, and pass through the head to become attached to the antero-dorsal rods of chitin of each side, near their origin from the transverse bar. The **protractor muscles** (*m. m.*) are attached to the antero-lateral walls of the frons, and pass upwards to become inserted along the base of the mandibular chitinous rods.

(I) The **retractor muscles of the maxillæ** (*m'. mx.*) are inserted at the proximal end of the maxillæ, and pass beneath the mandibular retractors to become attached to the antero-ventral rods of chitin, near the transverse bar.

The **protractor muscles** (*m. mx.*) are attached to the proximal ends of the maxillæ, and pass ventrally to the antero-ventral walls of the frons.

(3) The Musculature of the Ambulatory Appendages.—Two stout flexor muscles, which have their origin at the postero-dorsal margin of the mesothorax, are inserted on

the posterior face of the second pair of coxæ. Two similar flexor muscles extend from the post-dorsal margin of the metathorax to the third pair of coxæ.

A stout flexor and extensor muscle extend from the walls of each coxa to become inserted on the base of the trochanter.

A large extensor and a smaller flexor muscle extend throughout the femur. Inserted at the base of the tibia are a small flexor and extensor muscle, which continue throughout the article to the base of the tarsus.

(4) The Dorso-ventral Muscles of the Body.—Several bands of **dorso-ventral body muscles** extend from the floor of the thorax and abdomen to the dorsal and lateral walls of the body.

The **dorso-ventral muscles of the abdomen** (*m. v.*) arise from the ventral surface, close to, or a little external, to the outer longitudinal muscle bands, and pass upwards to become inserted on the dorso-lateral walls of the segments. It is these dorso-ventral muscles which bring about the respiratory movements of the body.

From the ventral surface of the seventh and eighth abdominal segments a band of muscles passes to each of the cornicles (*m. corn.*), and control the movements of the opening of those structures.

### The Pseudo-vitellus.

Situated in the posterior half of the abdomen, lying by the side of, or between the ovarian cæca, are a few roundish conspicuous cells, which are usually joined together in groups of two or three cells, or in young females may form two larger masses (fig. 45, *pr.*).

When stained in sections with hæmatoxylin and eosin, the cytoplasm of these cells readily takes the eosin stain and appears to be quite granular. In the centre of the cell is a deeply staining nucleus with a nucleolus, but in some cells the nucleus is not seen.

These cells form the so-called pseudovitellus of Huxley and

other authors, and the "Secundäre Dotter" described by Mecznikow (1866) in Coccidæ. Witlaczil (1882) has described them in *Aphis*, Krassiltschik (1892) in *Phylloxera*, Dreyfus (1894) in *Phylloxera*, Mordwilko (1895) in *Trama*, and Grove (1909) in *Siphonophora*; so that they appear to be generally present throughout the Aphididæ and probably the Coccidæ.

The origin and development of the pseudovitellus cells have been described by Will (1888) and also by Witlaczil (1884), but the function of these cells does not appear to be established. The appearance and distribution of them in the individual depends upon the stage of its development. In embryos of *S. lanigera* they form a conspicuous lobed mass of cells in the posterior region of the body. In mature, apterous viviparous females however, they degenerate into groups of two, three or more cells, lying to the side of the ovarian cæca in the posterior region of the body. I have observed in a few individuals, isolated pseudovitellus cells in the thoracic region of the body.

It would appear that these cells fulfil a nutritive function for the developing embryos, but Witlaczil (1882) considers them to be excretory in function.

Mordwilko (1895, p. 357) has briefly described the changes which take place in the pseudovitellus during the development of *Trama*. According to this author, the cells in young embryos of *Trama* form a tubular layer lying above the rectum, which, as the embryo develops, divides into three finger-like, longitudinal lobes, lying between the ovarian cæca. In mature adults only two cell masses are seen, which lie to the side of the body in the fifth and sixth segments, and these cells show signs of degeneration.

In addition to the pseudovitellus cells described above, there are in *S. lanigera* a few large cells situated in the posterior region of the body, which have a dirty-greyish, coarsely granular cell contents and a small nucleus (fig. 45, *y. c.*). When stained with hæmatoxylin and eosin they differ in appearance from the pseudovitellus cells, the cytoplasm

being a dirty-yellowish colour, taking the eosin stain poorly and containing numerous dark coarse granules.

Scattered throughout the body cavity are a few isolated roundish cells which are smaller than the pseudovitellus cells, and are found in the head, thorax and abdomen, but chiefly in the head and thorax (fig. 22, *a.*). They have a greyish granular cytoplasm and a comparatively large nucleus, and resemble in appearance the cells described by Dreyfus (1894, p. 232, fig. 13, *a.*), in *Phylloxera*.

### The Fat Body.

When examined in normal salt solution, the **fat body** is seen to consist of yellowish-brown masses of cells, which extend beneath the integument and over the internal organs. The cells are irregular in shape, much vacuolated, and possess numerous olive-coloured refractive granules and shining fat-globules.

As seen in sections, the fat body consists of several layers of cells, forming an irregular network beneath the integument and round the digestive tract. It is more extensively developed in the thorax and anterior segments of the abdomen, and is formed from the mesoblast cells lining the integument. In stained sections the cells of the fat body are seen to be irregular in shape with a much vacuolated cytoplasm and a small, irregular, stellate nucleus. The nucleus is surrounded by a layer of granular protoplasm and a similar layer extends round the periphery of the cell, strands of protoplasm passing across the cell between the two layers.

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## EXPLANATIONS OF PLATES 38-42,

Illustrating Mr. J. Davidson’s paper on “The Structure and Biology of *Schizoneura lanigera*, Hausmann or Woolly Aphis of the Apple Tree.—Part I: The Apterous Viviparous Female.”

### REFERENCE LETTERING.

*a.* Antenna. *a. d.* Antero-dorsal rod. *ab. i-ix.* Abdominal segments. *a. f.* Articulation of clypeus with epicranium. *a. g.* Abdominal ganglion. *an.* Anus. *ap.* Anal plate. *a. s. 1-7.* Abdominal spiracles. *a. t.* Anterior dorsal trachea. *a. v.* Antero-ventral rod. *b. c.* Buccal cavity. *b. i.* Body integument. *b. ta.* Basal joint of tarsus. *c.* Chitinous thickening at posterior end of buccal cavity. *cd.* Cylinder or “Kolben” of salivary pump. *c. i.* Integumental lip over opening of cornicle. *cl.* Claws. *cn.* Cornicle. *c. o.* Opening of cornicle. *cox.* Coxa. *cs.* Commissure. *c. t.* Connective tissue. *c. tr.* Stout trachea connecting thoracic spiracles. *cy.* Cytoplasm. *d. b.* Dorsal tracheal branch. *d. f.* Dorsal wall of

clypeus. *d. h.* Dorsal wall of head. *d. t.* Dorsal longitudinal trachea.  
*e.* Embryos. *e. c.* Epithelial cells. *e. s.* Epithelial cells of stomach. *f. a.*  
 Integumental areas of wax-glands. *fac.* Facets of the eyes. *f. b.* Fat  
 body. *fe.* Femur. *fr.* Clypeus. *g.* Ganglion. *g. c.* Ganglion cells. *g. o.*  
 Genital orifice. *g. p.* Genital plate. *g. s.* Granules of secretion. *h.*  
 Hypopharynx. *hd.* Head. *hr.* Integumental hairs. *hs.* Hypodermis.  
*i.* Intestine. *i. h.* Infra-posterior wall of head. *in.* Intima. *lbr.* Labrum.  
*l. g.* Longitudinal proboscis groove. *lr. g.* Longitudinal groove on  
 labrum. *l. t.* Ligament of connective tissue. *lu.* Lumen. *lu. s.* Lumen  
 of stomach. *m.* Large pump muscle. *m. a.* Depressor muscles of  
 antenna. *m. a'.* Elevator muscles of antennæ. *m. b.* Depressor muscles  
 of transverse bar. *m. b'.* Elevator muscles of transverse bar. *m. c.*  
 Circular muscles. *m. ca.* Muscles controlling cornicles. *m. cox.* Coxal  
 muscles. *m. d.* External dorsal longitudinal muscles. *m. d'.* Internal  
 dorsal longitudinal muscles. *m. f.* Lateral muscle of clypeus. *m. g.*  
 Mid-gut. *m. h.* Depressor muscles of head. *m. l.* Longitudinal muscles.  
*m. m.* Protractor muscles of mandibular setæ. *m'. m.* Retractor muscles  
 of mandibular setæ. *m. mx.* Protractor muscles of maxillary setæ.  
*m'. mx.* Retractor muscles of maxillary setæ. *m. p.* Lateral divaricator  
 muscles to the wall of clypeus. *m. ph.* Divaricator muscles of pharynx.  
*m. pr.* Proboscis muscles. *m. r.* Mandibular chitinous rods. *m. s.*  
 Metathoracic spiracle. *m. t.* Dorso-ventral muscles of thorax. *m. v.*  
 External ventral longitudinal muscles. *m. v'.* Internal ventral longi-  
 tudinal muscles. *m. v. d.* Dorso-ventral muscles. *ma.* Metathorax.  
*ma. g.* Metathoracic ganglion. *md.* Mandibular setæ. *md. o.* Mandi-  
 bular organs. *mo.* Mesothorax. *mo. g.* Mesothoracic ganglion. *mx.*  
 Maxillary setæ. *mx. o.* Maxillary organs. *mx. r.* Maxillary chitinous  
 rods. *n.* Nucleus. *no.* Nucleolus. *n. a.* Antennal nerve. *n. l.* 1-3. Leg  
 nerves. *n. m.* Nerve to muscles. *o.* Outer wall of œsophageal valve.  
*œs.* Œsophagus. *oc.* The eyes. *oc. n.* Nerve to eyes. *od.* Oviducts.  
*om.* Pigment. *p.* Post-abdominal nerve. *p. a.* Ovarian chambers  
*p. c.* Ovarian cæca. *p. g.* Post-abdominal ganglion. *ph.* Pharynx.  
*p. m.* Pump muscles attached to *v. r.* *po.* Prothorax. *p. p.* Pharynx  
 protuberances. *pr.* Proboscis. *p. s.* Prothoracic spiracle. *pv.*  
 Pseudovitellic cells. *r.* Rectum. *r. o.* Retort-shaped organs of embryo.  
*s.* Salivary pump. *s. a.* Anterior salivary gland. *sb. g.* Sub-œsophageal  
 ganglion. *s. c.* Salivary pump chamber. *s. d.* Salivary duct. *s. g.*  
 Supra-œsophageal ganglion. *s. o.* Antennal sense-organs. *s. p.* Posterior  
 salivary gland. *s. p.* Spiracle. *s. r.* Main trachea from spiracle. *st.*  
 Stomach. *seg. n.* 1, 2. Segmental nerves from *p.* *t.* Tactile hairs. *ta.*  
 Tarsus. *t. g.* Thoracic ganglion. *ti.* Tibia. *tr.* Trachea. *tro.* Trochanter.  
*t. t.* Thoracic trachea joining the spiracles. *v.* Vagina. *v. b.* Ventral  
 tracheal branch. *v. l.* Ventral longitudinal trachea. *v. r.* Ventral

chitinous rods. *w.* Wall of the ovarian chamber. *w. c.* Wax-gland cells. *w. f.* Wall of clypeus. *w. gl.* Wax-gland. *w. h.* Wall of head. *w. s.* Wax-sac. *w. s'.* Solidified contents of wax-sac. *x.* Cells described in text. *y. c.* Large cells with coarse granular contents.

## PLATE 38.

Fig. 1.—Apterous viviparous ♀, ventral. × 45.

Fig. 2.—Apterous viviparous ♀, dorsal. × 45. 2 B. Wax-glands showing integumental facettes. 2 c. Eyes showing tubercles.

Fig. 3.—Head, anterior portion (frons), showing endoskeleton as seen from dorsal or anterior aspect. × 375.

Fig. 4.—Appendages: (A) Leg 1; (B) leg 2; (c) leg 3. × 85. (D) antenna (right) ventral side. × 110.

Fig. 5.—Apterous viviparous ♀, dissection from dorsal surface showing distribution of internal organs; the dorsal integument, dorsal longitudinal muscles and tracheal system removed. × 85.

## PLATE 39.

Figs. 6-21.—Series of transverse sections through the head, being at right angles to its longer axis, showing relationship of endoskeleton, pharynx, buccal appendages and musculature. × 250.

Fig. 22.—Longitudinal section through side of the head. × 135.

Fig. 23.—Longitudinal section through head, passing through the end of the transverse bar. × 135.

Fig. 24.—Longitudinal section through head and thorax, almost median. × 235.

## PLATE 40.

Figs. 25-32.—Series of vertical sections through head, showing relation of parts of endoskeleton, buccal appendages and musculature. × 200.

Fig. 33.—Vertical section through head showing salivary pump, etc. × 375.

Fig. 34.—Transverse section through thorax showing retracted proboscis. × 200.

## PLATE 41.

Fig. 35.—Apterous viviparous ♀, internal organs from right side, slightly schematised. Drawing made from dissections and serial sections. × 100.

Fig. 36.—Nervous system and salivary glands, dissection from ventral surface.  $\times 110$ .

Fig. 37.—Transverse section through the rectum; hæmatoxylin (Ehrlich) and eosin.  $\times 710$ .

Fig. 38.—Transverse section through small intestine; hæmatoxylin (Ehrlich) and eosin.  $\times 710$ .

Fig. 39.—Apterous viviparous ♀, dorsal, showing dorsal longitudinal muscles and dorsal tracheal system.  $\times 68$ .

Fig. 40.—Transverse section through stomach in region of œsophageal valve; hæmatoxylin and eosin.  $\times 710$ .

Fig. 41.—Longitudinal section through salivary glands; hæmatoxylin and eosin.  $\times 780$ .

Fig. 42.—Transverse section through the vagina; hæmatoxylin and eosin.  $\times 710$ .

#### PLATE 42.

Figs. 43-46.—Transverse sections through the body; hæmatoxylin and eosin.  $\times 135$ .

Fig. 43.—Transverse section through region of anterior salivary glands.

Fig. 44.—Transverse section through region of posterior salivary glands, showing salivary duct passing beneath the ganglion.

Fig. 45.—Transverse section through anterior segment of abdomen.

Fig. 46.—Transverse section through region of cornicles.

Fig. 47.—Section through the unicellular wax-glands; hæmatoxylin and eosin.  $\times 1100$ .

Fig. 48.—Longitudinal section through the stomach and œsophageal valve; hæmatoxylin and eosin.  $\times 780$ .

Fig. 49.—Section through one of the lateral eyes; stained hæmatoxylin and eosin.  $\times 780$ . The hypodermis has shrunk away from the chitin.

#### APPENDIX.

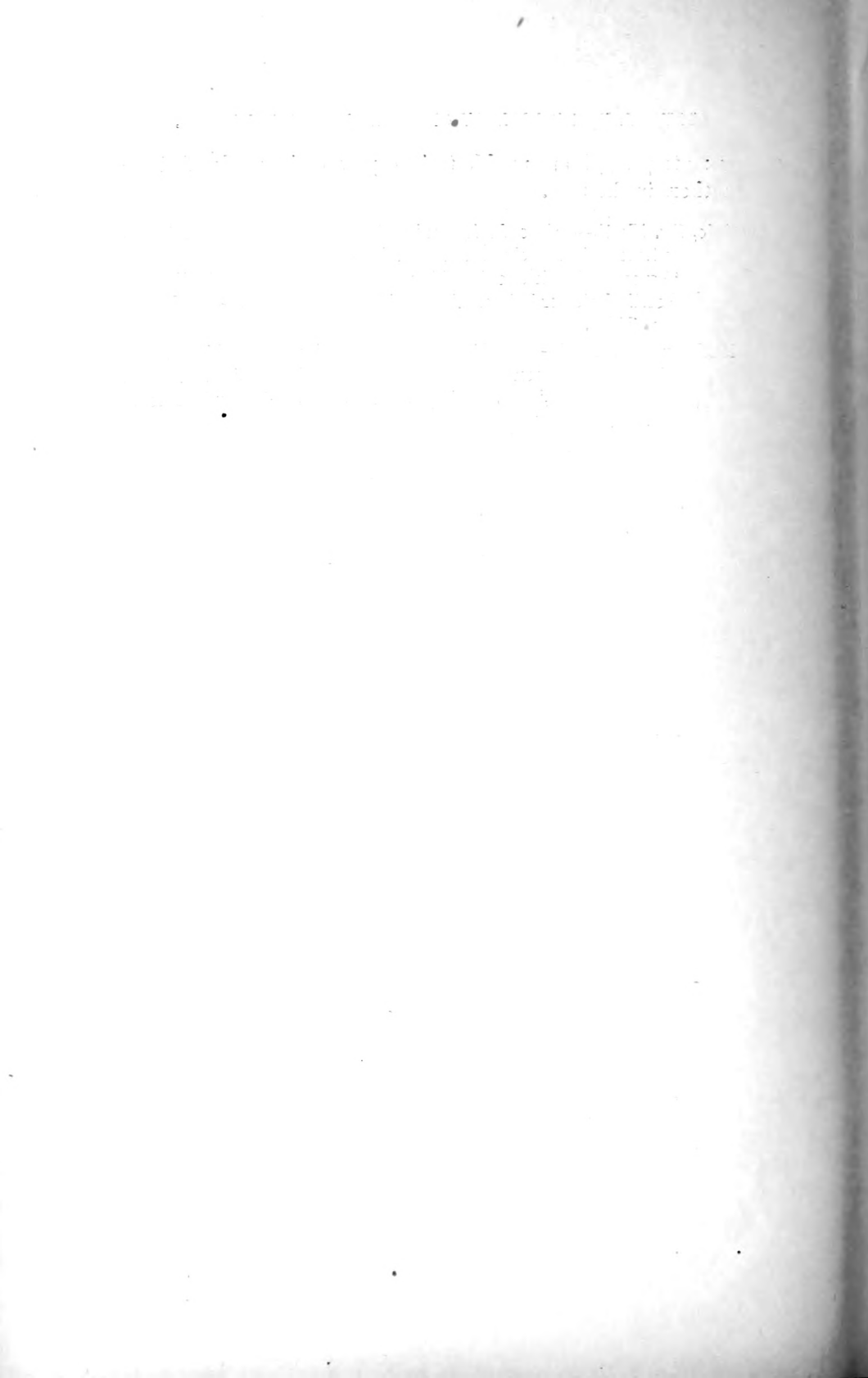
Through the kindness of Mr. Clifford Dobell my attention has been directed to the recent works of Dr. Karel Šulc and Dr. Paul Buchner on the pseudovitellus in Hemiptera. I regret that owing to the fact that the manuscript had already



gone to press, I am unable to incorporate the work of these authors in the text.

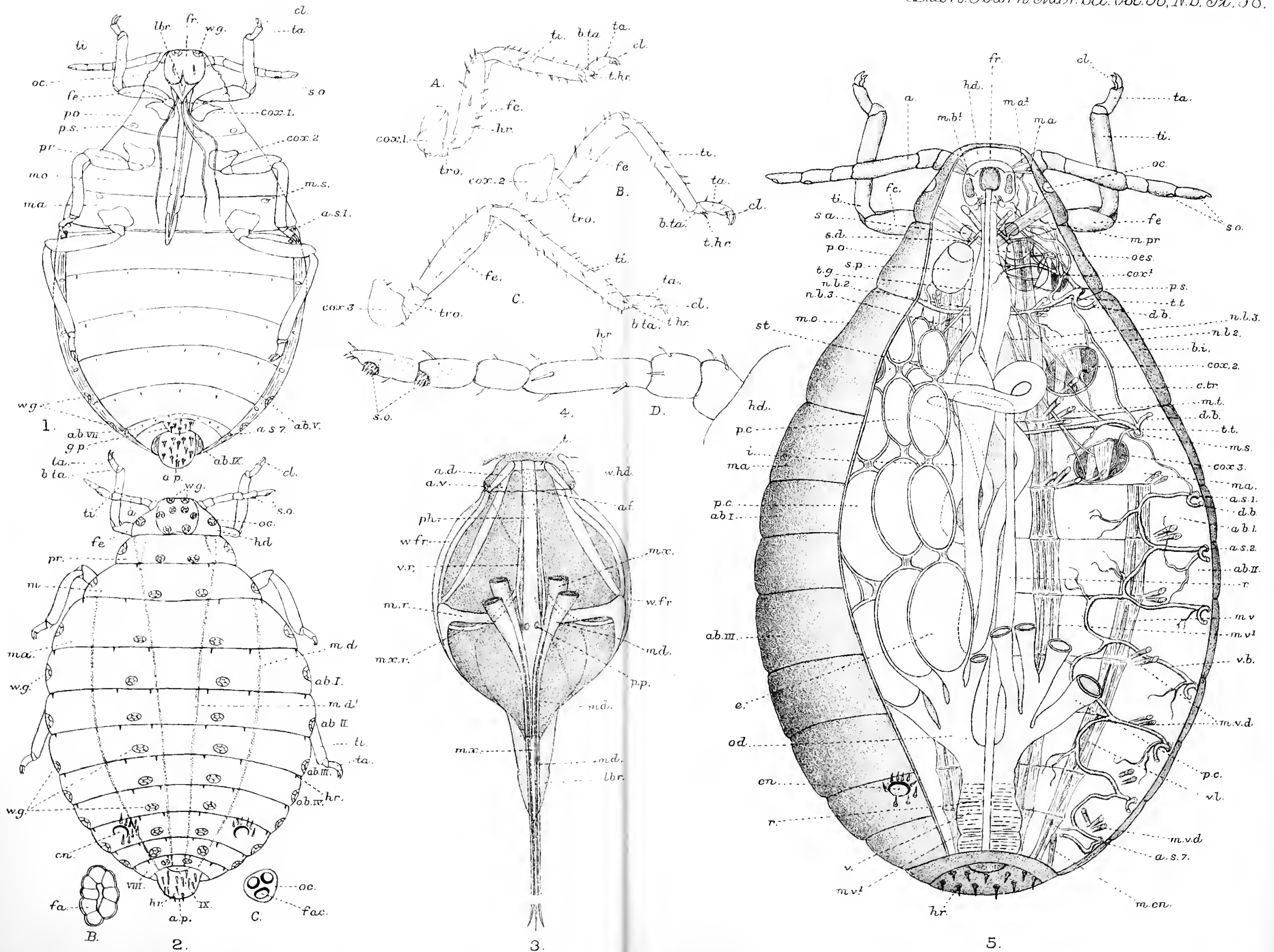
Šulc, Dr. Karel.—“‘Pseudovitellus’ und ähnliche Gewebe der Homopteren sind wohnstätten symbiotischer Saccharomyceten,” ‘Separatabdruck aus den Sitzungsberichten der Königl. Böhm. Gesellschaft der Wissenschaften in Prag,’ 1910, pp. 1-39, 18 text-figs. (1910).

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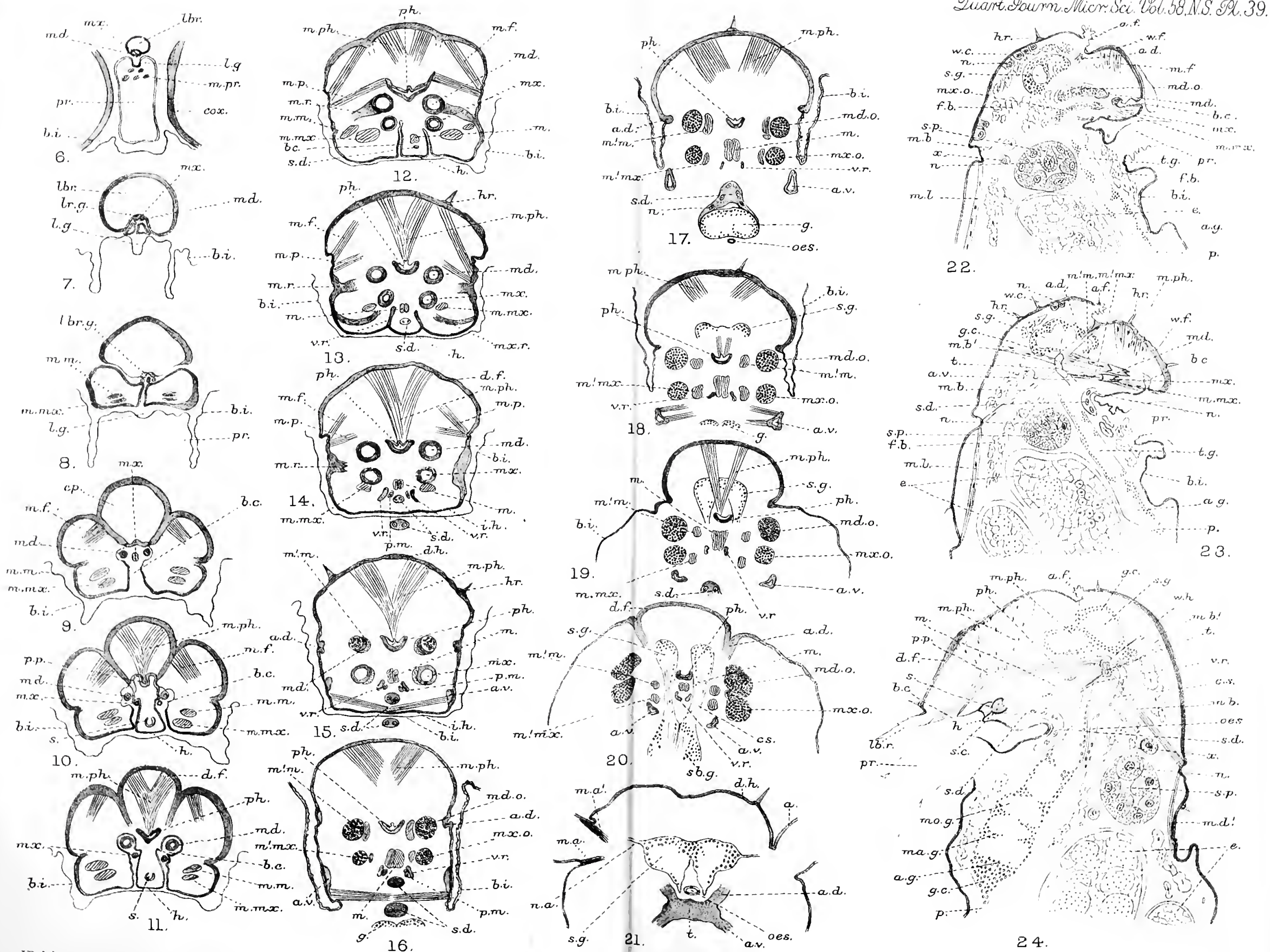


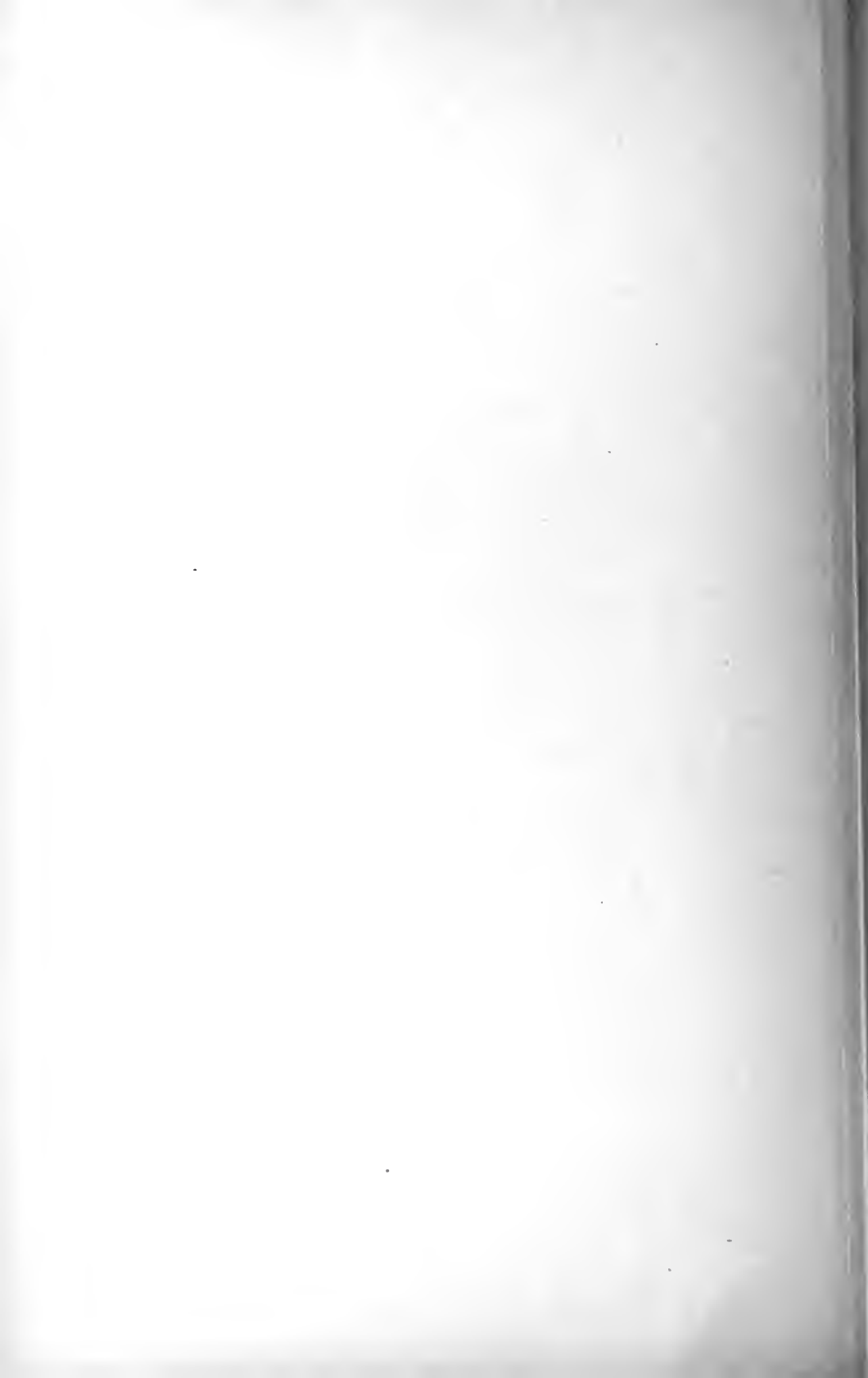








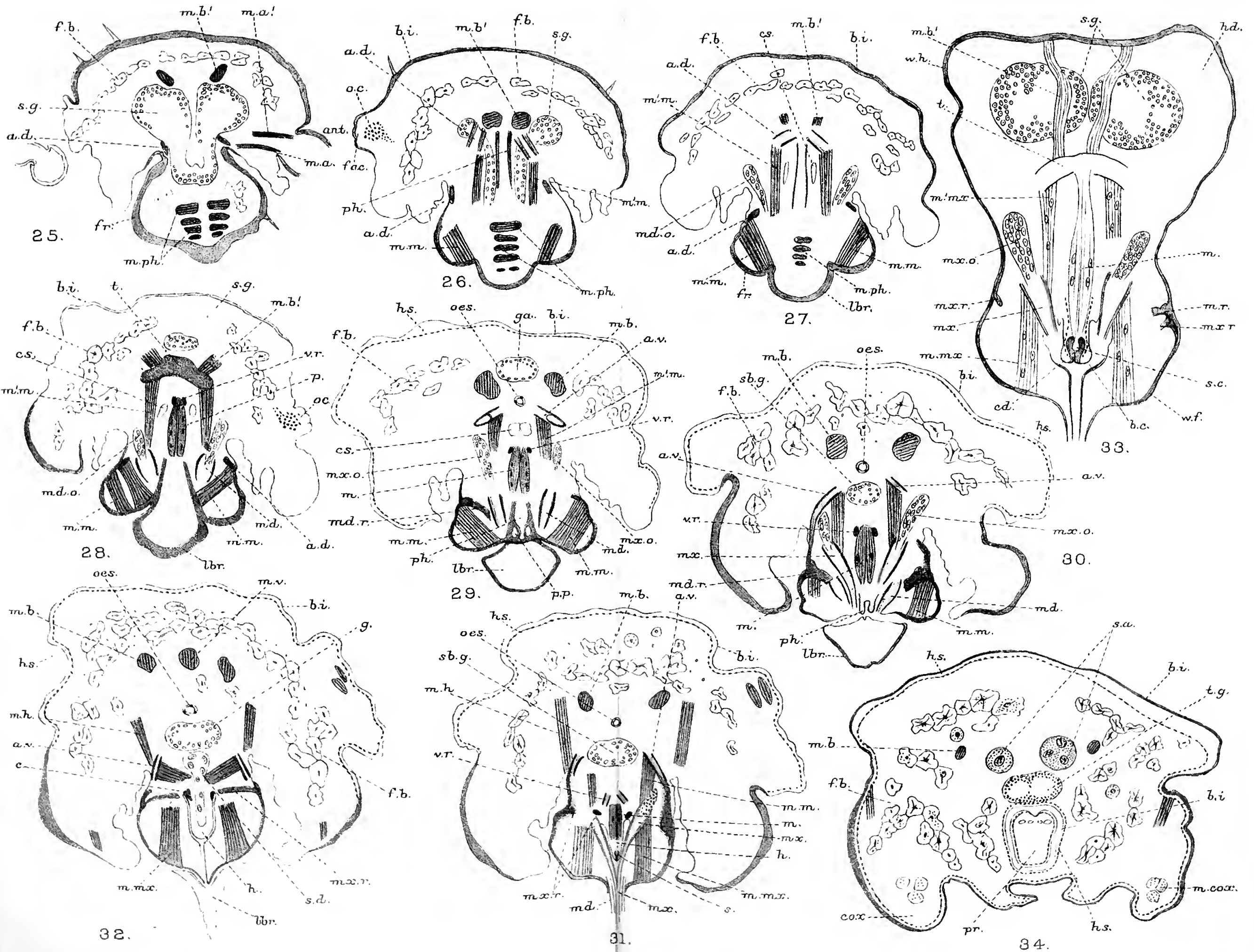






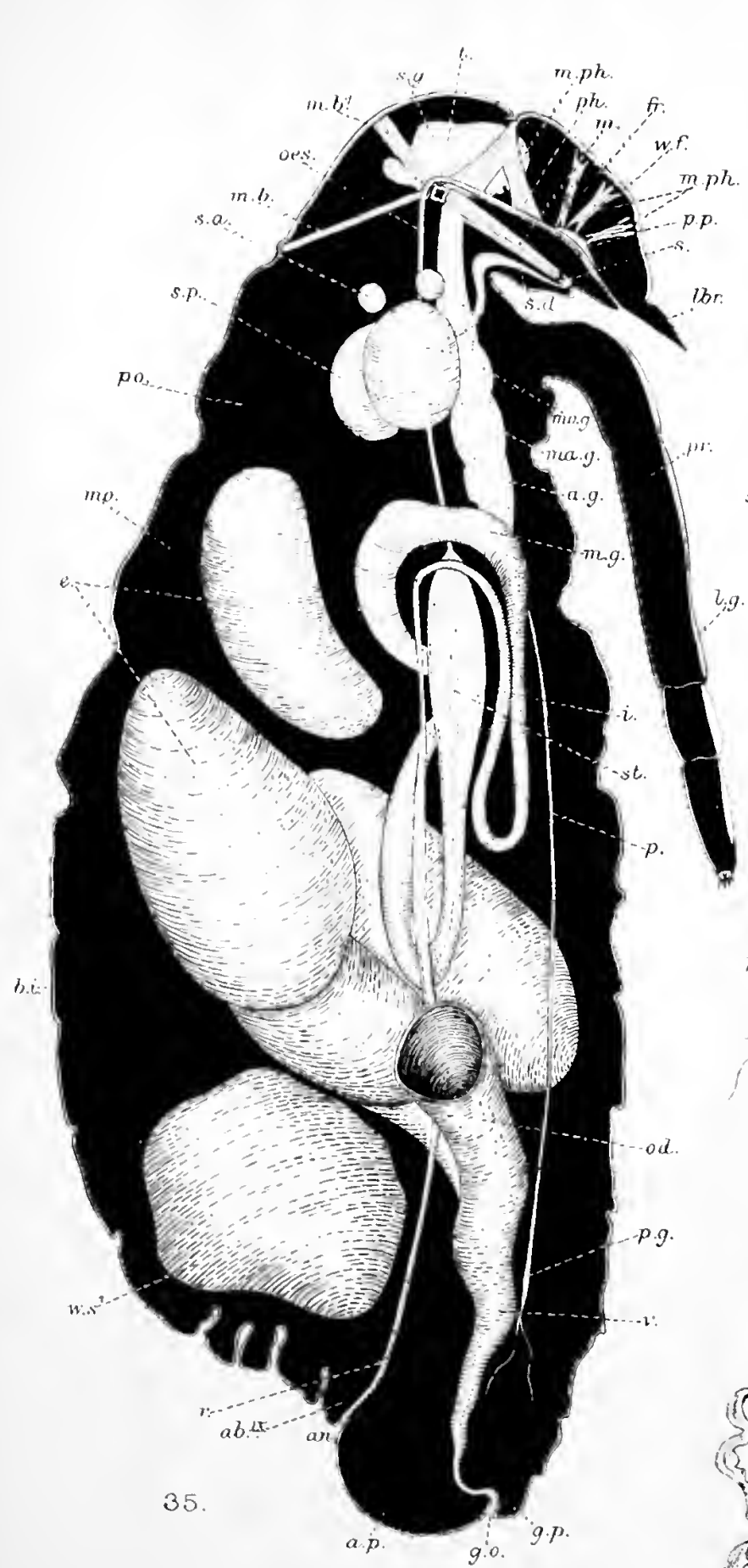




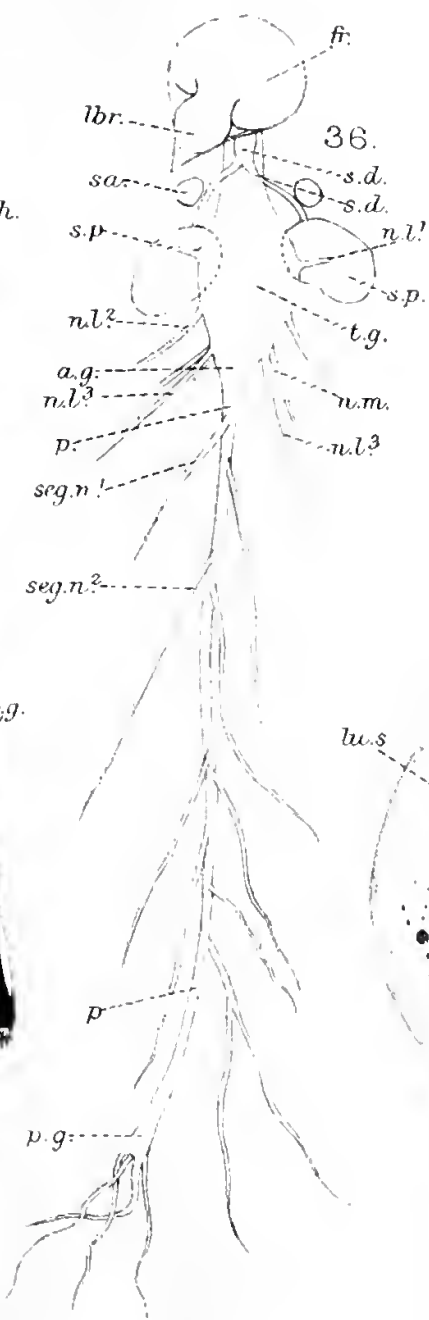




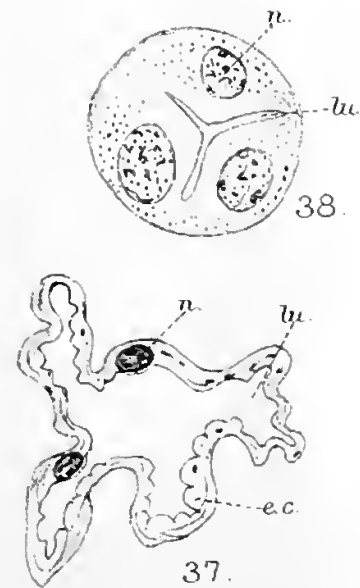




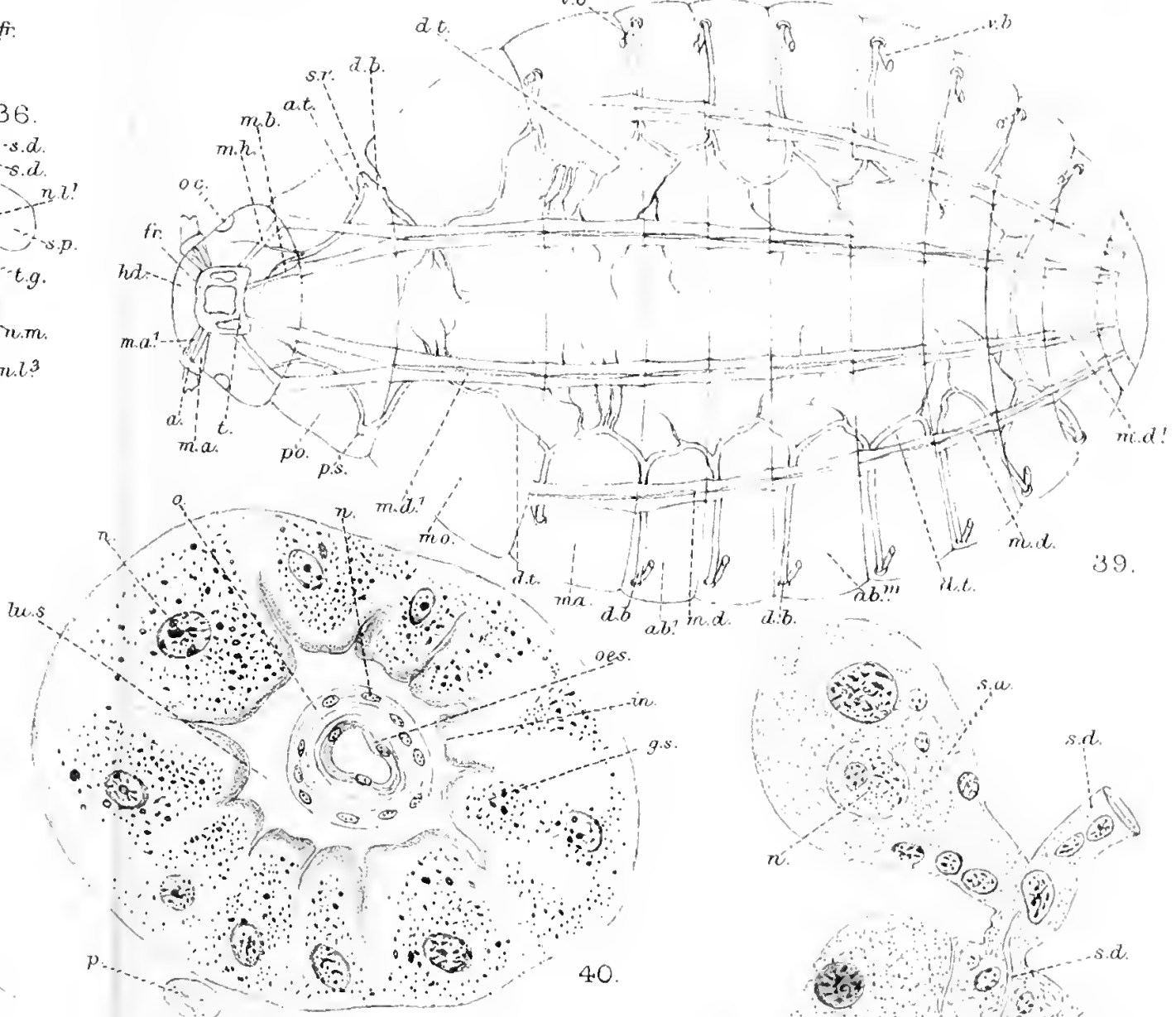
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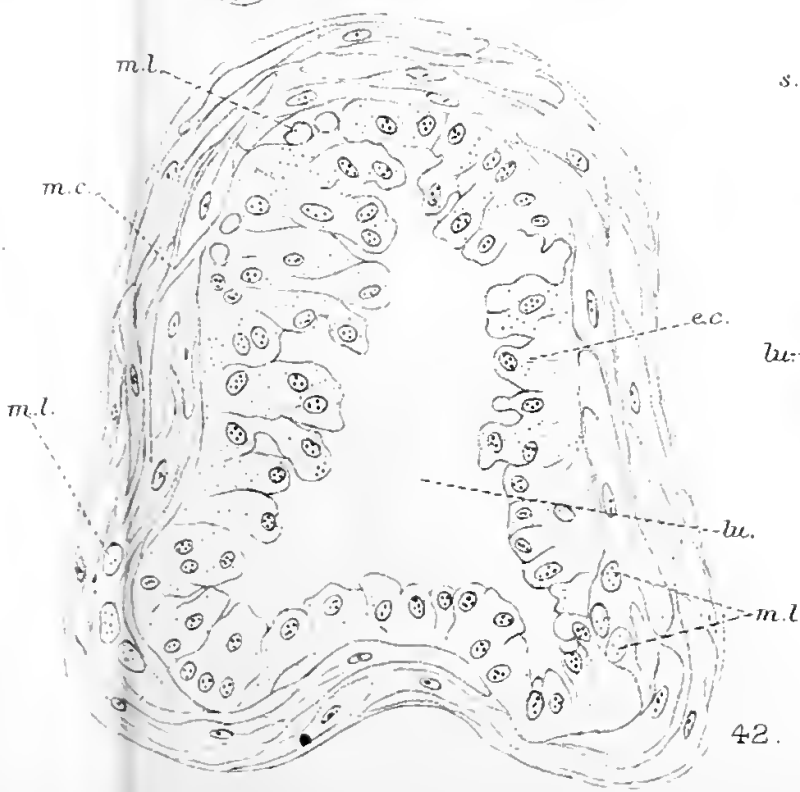
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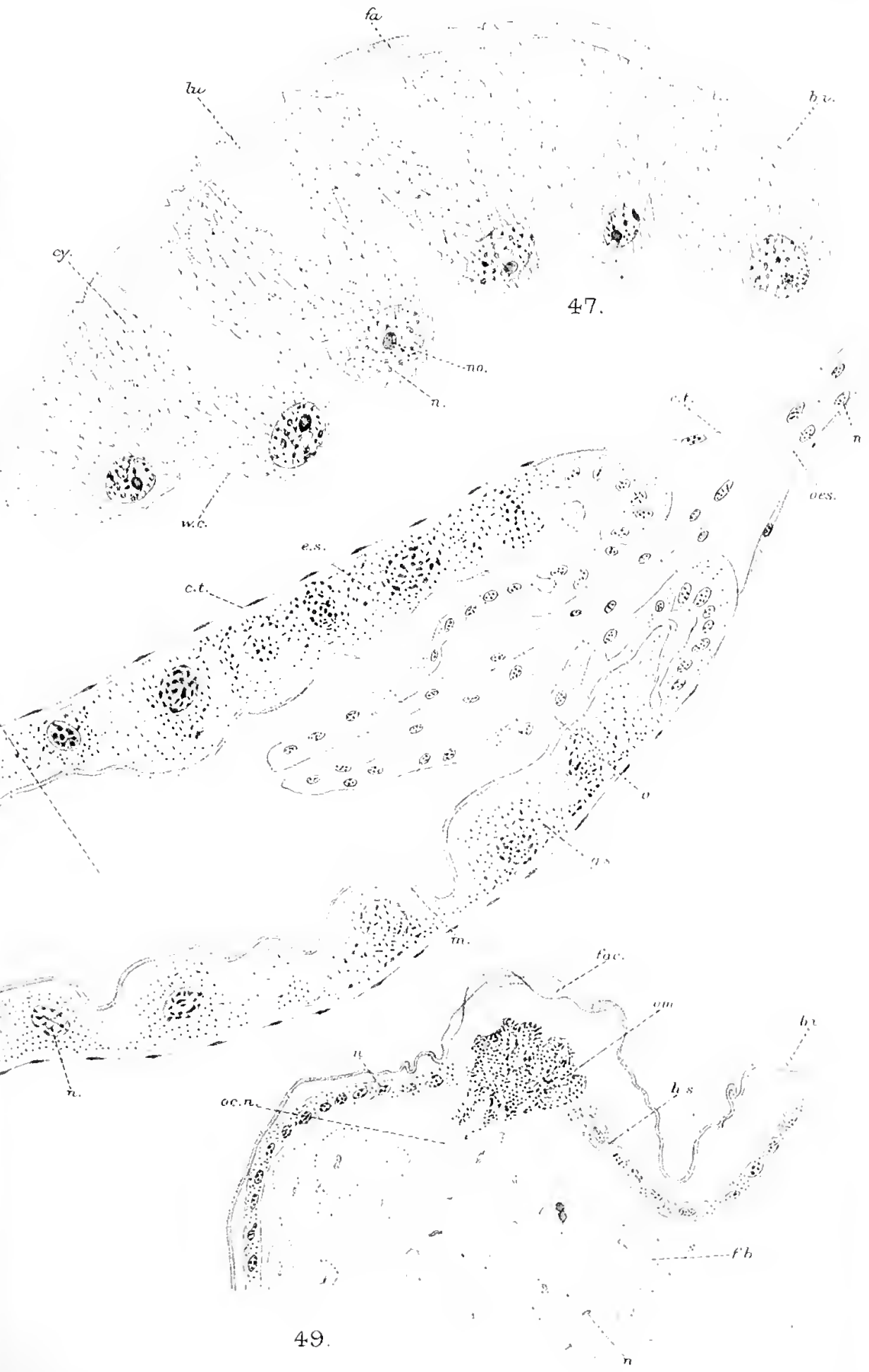
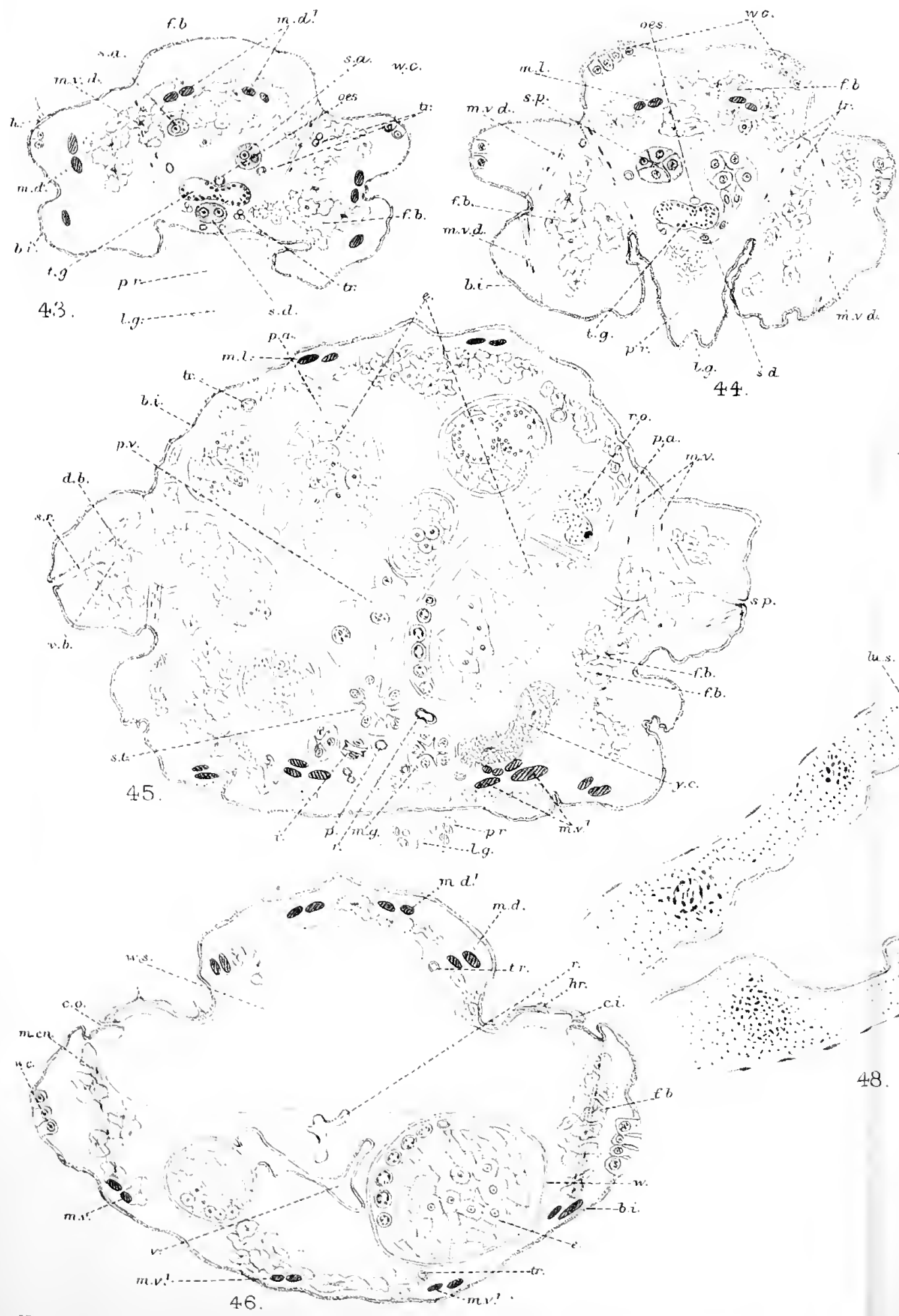
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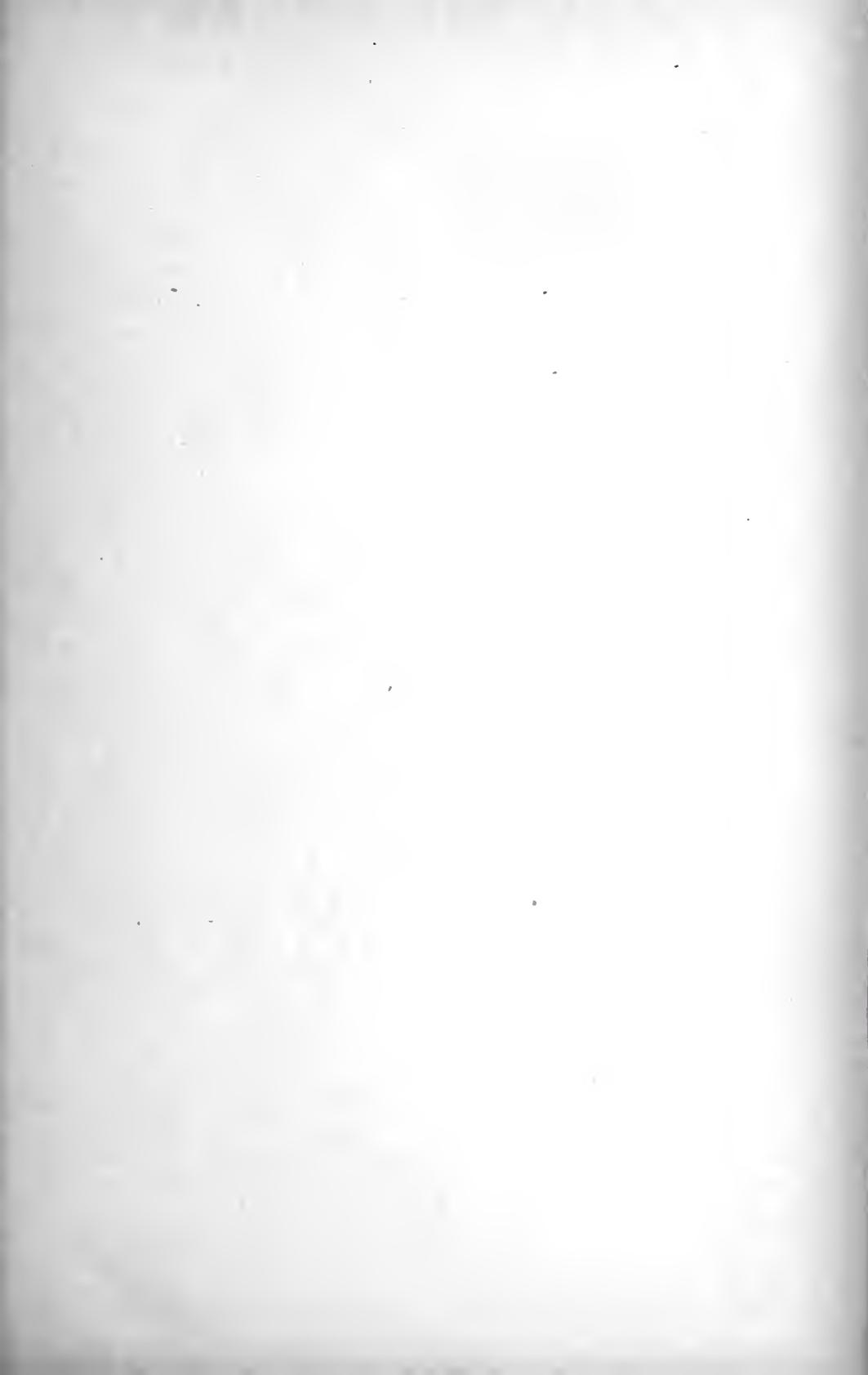
















**An Account of the Anatomy and Homology of  
the Adipose Lobe of the Pelvic Fin of the  
Salmon.**

By

**Edward W. Shann, B.Sc.,**

Demonstrator and Assistant Lecturer in Zoology in the Victoria  
University of Manchester.

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With Plate 43 and 3 Text-figures.

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I. INTRODUCTORY.

DURING the past two years the Zoological Department in Manchester University has been in receipt of fresh salmon, mainly from the Wye, which have been sent for the purpose of age determination by means of scale-markings. In December last Professor Hickson called my attention to the presence of a fleshy lobe lying immediately above the pelvic fin of a large female specimen. As we did not then know whether this growth was normal, and, if normal, whether it was confined to female specimens, I undertook to investigate the question; the investigation has not only proved exceedingly

interesting, but has opened up a wider scope of research than I had anticipated.

On consulting various books on fishes I found that the lobe was invariably figured in the best drawings of the salmon; in Day's 'British Fishes,' for instance, it can clearly be seen.<sup>1</sup> At the same time no reference to the occurrence of such a lobe could be found in any book which might be expected to throw light upon a structure of this nature. I made a dissection of the adipose lobe, and so found that it was supported at its base by a splint of bone; the posterior, or distal, extremity of this bone was united to, but not fused with, the outermost fin-ray. This splint of bone is depicted in a figure of the pelvic fin skeleton of the trout in Parker and Haswell's 'Text-book of Zoology,'<sup>2</sup> and it is there mentioned that "the adipose lobe of the pelvic fin is supported by a small scale-like bone." By treatment of sections of the lobe with osmic acid and with Sudan III, I was able to satisfy myself that a considerable amount of fat was present in the lobe; I further observed from my sections that the lobe was stiffened by a plate of hyaline substance which ran throughout its length. I was fortunate enough to obtain a series of young salmon ranging in age from one to five months; from these I prepared serial sections of the pelvic region, and was thereby enabled to observe the origin and growth of the adipose lobe. From these observations I have been able to demonstrate that this so-called "adipose lobe" is nothing more or less than an enlarged scale which has never pierced its connective-tissue pocket. That a scale is capable of becoming a fin-like structure is an additional support to Mr. Goodrich's hypothesis, namely, that the dermal fin-rays (lepidotrichia) are modified body-scales, if his lucid argument has not already gained general acceptance.

I further examined the condition of the pelvic fins of other Teleosteans; and my observations from illustrations and from actual specimens go to show that an enlarged scale in the

<sup>1</sup> See also fig. 1 herein.

<sup>2</sup> Ed. 2, vol. ii, fig. 864.

outer angle of the pelvic fin is a character which is common to a wide range of members of that class. This "accessory scale," if I may so term it, is particularly characteristic of the more primitive Teleosteans, and is almost invariably absent in highly specialised forms; it is often well developed in actively swimming forms, and absent, or much reduced, in forms which haunt the ground. Only in the genus *Salmo*, so far as I am aware, is the accessory scale converted into an adipose lobe, and only in this genus, and perhaps in *Coregonus*, has it any skeletal connection with the fin-rays of the pelvic fin.

The nature of the function which this adipose lobe serves is obscure, but it is possible that it may assist in facilitating the rapid movements for which this fish is noted. The subject will be treated more fully at the conclusion of this paper.

I wish to acknowledge my indebtedness to Miss P. C. Esdaile, M.Sc., for permission to remove the portions of the salmon (which had been supplied by J. A. Hutton, Esq., for work upon which she was engaged) which I required, also for furnishing me with various particulars as to the size, age, locality and condition of the several specimens. To Professor Lorrain Smith I am indebted for the use of apparatus in the Pathological Department, and for his ready assistance in determining the nature of the fat in the adipose fins. Finally, I am glad of this opportunity gratefully to acknowledge the kindness of Professor J. W. Spengel for granting me a table for work in his laboratory at Giessen, and for the use of apparatus and materials; also that of Professor J. Versluys for his friendly interest and for many illuminative suggestions.

Figs. 1 and 2 illustrating this work were prepared from photographs kindly undertaken respectively by Mr. J. T. Wadsworth, of the Zoological Department at Manchester University, and by Mr. A. W. Brown, of the Gatty Marine Laboratory at St. Andrews.

## II. DETAILS OF THE ANATOMY OF THE PELVIC FIN AND OF ITS ADIPOSE LOBE.

### (1) Anatomy of the Pelvic Fin.

The anatomy and development of the pelvic fin of the salmon has been described in a paper by K. G. Harrison,<sup>1</sup> and, as it is desirable that the environment of the adipose lobe should first be realised, I give below Harrison's summary of the anatomy:

“Die Lage der Bauchflosse variirt bekanntlich sehr stark bei den verschiedenen Teleostiern. Sie kann hinter, direkt unter, oder vor der Brustflosse liegen und wird dementsprechend bauch-, brust-, oder Kehlständig genannt. Die erste dieser Lagen ist natürlich als die ursprüngliche anzusehen. Diese findet sich bei dem Lachs. Diese Flosse ist an der ventralen Seite des Körpers gelegen, sehr nahe zur Medianebene, und ganz ventral von der Rumpfmuskulatur. Die Muskeln der beiden Flossen werden zum grössten Theil nur durch ein dünnes Lager Fettgewebe von einander getrennt. Kopfwärts ragt zwischen sie auf eine gewisse Strecke der *M. rectus abdominis* hinein.

“Die Flossenstrahlen sind am Körper in einer schrägen Linie befestigt. Die Basen beider Flossen convirgiren somit caudalwärts gegen die Mittellinie. Der grosste Theil des primären Skeletes der Flosse besteht vorzugsweise aus einem Knochen, der als *Basale metapterygii* bezeichnet wird. Derselbe ist dreieckig und nur theilweise verknöchert. Die caudale Seite des Dreiecks ist die Kürzeste; an ihm articuliren vermittelst kleiner Radien die Flossenstrahlen. Die Länge des Knochens übertrifft seine Breite um ein bedeutendes und er dehnt sich, wie auch die Muskeln, weit von der Basis der freien Flosse nach vorn aus.

“Die Ebenen, in denen diese Knochen auf den beiden Seiten des Körpers liegen, convergiren dorsalwärts. Daher

<sup>1</sup> Harrison, K. G., “Unpaar. u. Paar. Flossen. d. Teleostien,” ‘Arch. für Mikr. Anat.’ 1895, p. 530.

liegen die *Mm. Adductores* beinahe ganz dorsal zum Knochen eingebettet zwischen Hartgebilden und Muskeln des Rumpfes, während die *Abductores* zum grössten Theil ventral vom Flossenskelet sich finden.

“Der *M. abductor superficialis* entspringt von der Fascie, welche den tiefliegenden bedeckt und zieht zu der Basis eines jeden Flossenstrahles. Er ist der kleinste von allen Flossenmuskeln.

“Der *M. abductor profundus* entspringt von der medianen Kante des Basale und befestigt sich an der Innenseite der ventralen Strahlenhälfte.

“Der *M. adductor superficialis* ist leicht in zwei Portionen trennbar. Ein laugfaseriger Theil entspringt von dem oralen Ende des Basale und inserirt an den vier antero-lateralen Strahlen. Ein Kursfaseriger Theil ist mehr oder weniger deutlich in einzelne. Muskelbündel zerlegt, die von der Fascie der Körpermuskulatur entspringen und an der Basis der caudalen Strahlen inseriren. Der Verlauf der letzten caudal gelegenen Muskelbündel ist beinahe vertical.

“Der *M. adductor profundus* entspringt vorzugsweise von der medianen Kante des Basale und ist symmetrisch zum entsprechenden Bündel des *Abductor profundus* an der Innenseite der dorsalen Strahlenhälfte befestigt. Der Muskel übertrifft an Masse den oberflächlichen *Adductor* um ein Bedeutendes.

“Die Nerven der Flosse bilden einen Plexus und stammen von sechs Rückenmarksnerven ab.”

My observations bear out in every respect the above account of the musculature of the pelvic fin of the salmon, and I will only add that, in adult specimens, the tendons fastened to the bases of the fin-rays are very distinct; forwards, however, the muscle sheets are quite continuous, excepting that of the *adductor superficialis*, which, as Harrison remarks, is divided longitudinally into two portions. There are normally nine fin-rays in each pelvic fin, and though most authors quote ten as a variation from the normal, I have not yet met with this condition.

In addition to the bony elements of the pelvic fin, namely the Basale metapterygii and the lepidotrichia (dermal fin-rays), which have been mentioned above, there are in addition three small nodules, the distal pterygiophores (see fig. 2, *Pt.*). The precise position of these nodules varies not only in different specimens, but in the right and left fins of the same specimen. The largest pterygiophore is placed invariably, so far as my observations go, between the dorsal and ventral halves of the ninth, or inner, fin-ray. The middle pterygiophore varies in position, but not infrequently occurs between the dorsal and ventral halves of the fourth fin-ray. The outer pterygiophore occurs between the dorsal and ventral halves of the third or second fin-ray. In one specimen the middle and outer pterygiophores were bound closely together and occupied the space between the third and second fin-rays. On warming each of these pterygiophores in turn in a weak solution of caustic potash, the inner was found to remain single, but the middle and outer split fairly readily each into two distinct nodules. That all these several portions are ossified is indicated by the fact that they effervesce when placed in dilute hydrochloric acid.

Having now in mind the position and essential points in the anatomy of the pelvic fin of the salmon we shall proceed to locate and to describe the structure, known as the adipose lobe, with which it forms an intimate connection.

## (2) Anatomy of the Adipose Lobe.

The lobe (fig. 1) lies in the angle between the pelvic fin and the body-wall in a position dorsal to and abaxial from the first, or outermost, fin-ray. It presents a vertical triangular surface when the fish is viewed from the lateral aspect, the acute-angled apex of the triangle being directed posteriorly; at the proximal end its section is approximately in the form of an equilateral triangle, whose apex is directed inwards and is united to the body-wall of the fish; at the aboral end the lobe is free and plano-convex in section, the convex



side is adaxial and simply represents the result of the gradual rounding-off of the angular adaxial surface which has been noted at the proximal end. The outer vertical surface is grey in colour and firm in texture; the two inner surfaces, namely the dorso-lateral which faces the body-wall, and the ventro-lateral which faces the upper surface of the outermost fin-ray, are white in colour and soft to the touch. When at rest, the two inner surfaces of the lobe are closely apposed to the surfaces of the fish which they respectively subtend; as a result of this the soft ventro-lateral surface is grooved in its posterior half owing to the pressure of the fin-skeleton.

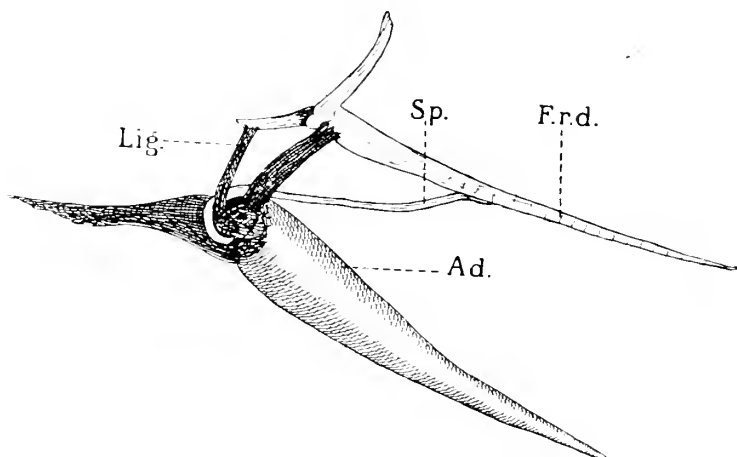
The lobe is equally well developed in specimens of both sexes, and, as far as one can judge from the comparatively few specimens which I have been able to examine, it further appears that the size of the lobe is not altered during the seasonal movements of the fish. Specimens were examined in the pink of condition, fresh from the sea, and others which had spawned and were, in some cases, infected with fungus, but neither the regular outline of the lobe nor its consistency appeared to differ in any degree; this is worthy of note, as it is well known that the body-scales of the salmon undergo a very marked disintegration at their margins, known as the spawning-mark, after the sexual products have been shed. Only in one unfortunate fish, which had long been suffering from an unsightly wound,<sup>1</sup> was the adipose lobe, on either side, reduced to a mere stump. The following measurements are from a male salmon weighing 22½ lb., 100 cm. in length, 52 cm. in girth, taken in the Wye, and will serve to convey an idea of the normal proportions of the adipose lobe:

Length of outer margin of pelvic fin	. 10.0 cm.
,,    inner    ,,    ,,    .	. 5.5 ,,
Breadth of distal    ,,    ,,    .	. 6.0 ,,
,,    proximal  ,,    ,,    .	. 3.0 ,,
Length of adipose lobe    .	. 4.0 ,,
Breadth    ,,    (at base)	. 0.7 ,,

<sup>1</sup> A hole about an inch in diameter piercing the body-wall and entering the abdominal cavity in the anal region.

On removing the skin from the base of the adipose lobe and that covering the dorsal aspect of the outermost fin-ray a splint of bone, fig. 2 (*Sp.*), was observed connecting the fin-ray with the base of the adipose lobe. At its adaxial extremity this splint of bone is attached by connective tissue, just as two adjacent fin-rays are connected, to the dorsal half of the outermost fin-ray posterior to the point where the

TEXT-FIG. 1.



Adipose lobe from the left side to show its connection with the pelvic fin. The ligament connecting with the dorsal ramus of the head of the fin-ray has been severed, and the lobe deflected outwards through an angle of  $180^\circ$  (so that its inner, angular surface is presented). *Ad.* Adipose lobe. *F.r.d.* Dorsal half of the 1st fin-ray. *Lig.* Ligament. *Sp.* Splint of bone.

latter is joined by the ventral half; but while the two halves are still quite distinct, the splint in this region is rectilinear, and in girth somewhat more slender than the dorsal half of the fin-ray. At its abaxial extremity the splint curves sharply upwards so as to circumscribe the base of the adipose lobe (Text-fig. 1). There appear to be no muscles connected with the splint; it simply lies in a pocket of connective tissue, to the walls of which it is loosely attached; nor are there in the adult any muscles connected with the adipose lobe. On the

inner aspect of the latter, however, near the proximal extremity, there are certain masses of tough ligament (*lig.*), which unite it with the heads of the dorsal halves of the first (outermost) and second fin-rays. Owing to this arrangement a certain amount of movement of the adipose lobe is consequent to the movement of the fin-rays, but there appears to be no mechanism for independent action. Further dissection of the lobe revealed the presence of an irregularly shaped mass of hard substance running throughout its length; near the base the mass is more or less spear-shaped, so that it appears linear in cross section; as the distal extremity is approached the section becomes triangular, but in all parts a flat surface runs parallel with, and close beneath, the vertical outer wall of the lobe.

### (3) Histology of the Adipose Lobe.

Transverse sections, taken near the base of the lobe shortly before it loses all connection with the body-wall, are triangular in outline. Fig. 3 represents such a section together with the adjacent portion of the body-wall. The first feature of note is the entire absence of epidermis, not only on the surface of the lobe, but also on the surface of the opposing body-wall; this condition invariably obtained in the case of all adult specimens examined by me.<sup>1</sup> The lobe is bounded by a single strand of homogeneous material, which is slightly refringent and stains deeply with iron-hæmatoxylin; this strand I take to be the *membrana basale* (*M.b.*). Below the *membrana basale* the lobe is composed of dense connective tissue (*C.t.*), whose fibres run approximately

<sup>1</sup> It should be mentioned, however, that the fish had all travelled considerable distances before they reached me, so that the epidermis may have been rubbed off during transit or may have decomposed during the time that elapsed before the tissues were placed in the preserving fluid. I hope later to have an opportunity to clear this doubtful point by preserving carefully the adipose lobe from a salmon immediately it is taken from the water.

parallel to the surface of the lobe which they subtend. In the centre of the lobe there is a fatty tissue (*F.t.*); the connective tissue here is loosely and irregularly arranged to allow space for the fat-globules. The latter have been dissolved by the action of alcohol in the preparation depicted in fig. 3; they will be described later when fig. 5 is examined. The accessory scale (*Sc. A.*), can be seen as a band of refringent substance running parallel to the vertical outer wall of the lobe throughout its entire height. It is surrounded by a scale pocket (*Sc.p.*). A normal body-scale (*Sc.*) is also shown in fig. 3; on comparing the accessory scale with this, it is seen that the former is very much the larger, but does not show the characteristic concentric markings. In thicker sections from this region of the lobe a few large pigment-cells were observed embedded deep in the outer vertical wall.

Fig. 4 represents a transverse section taken through the base of the adipose lobe quite close to its origin, where it is scarcely to be distinguished externally from the body-wall. Two scales (*S.c.*) are seen lying in their pockets (*Sc.p.*), close beneath the outer wall. The accessory scale (*Sc.a.*) is cut close to its root, and appears shorter and thicker than in the section shown in fig. 3; the arrangement of the tissues is similar in the two sections. Near the inner aspect a round space is seen (*Sp.h.*); this is due to the removal of the bony splint (Text-fig. 1, *Sp.*) which supports the adipose lobe. It was noticed above, under the description of the dissection of the lobe, that the splint lay in a pocket of connective tissue; now in the section, the hole (*Sp.h.*) is seen to be surrounded by concentric stands of connective tissue, which give it the appearance of a scale-pocket. This resemblance will receive further notice in the section on homology.

Proceeding towards the middle of the length of the lobe, where it is entirely free from the body-wall, we obtain a cross section which is still roughly triangular; such a section is shown in fig. 5. The outer vertical wall (*O.*), is approximately straight, the dorsal angle (*D.*) is acute, the ventral

extremity (*V.*) has become flattened, the dorso-lateral border is markedly longer than the ventro-lateral, the latter is deeply furrowed. There is no epidermis; the outer border is, as before, formed by the *membrana basale* (*M. b.*) Immediately below the surface occur several layers of closely packed connective-tissue fibres. These run parallel to the surface which they subtend, and are very definitely defined; they are not shown in fig. 5, which was drawn from a hand-cut section; they appear, however, in fig. 6. There is a large accumulation of fat-globules (*Ft.*) in the central area; they appear as a band of orange running parallel with the accessory scale in sections stained with Sudan III. In addition a small clump appears in the central area on the outer side of the scale, where the latter makes a small bend away from the outer border of the lobe.

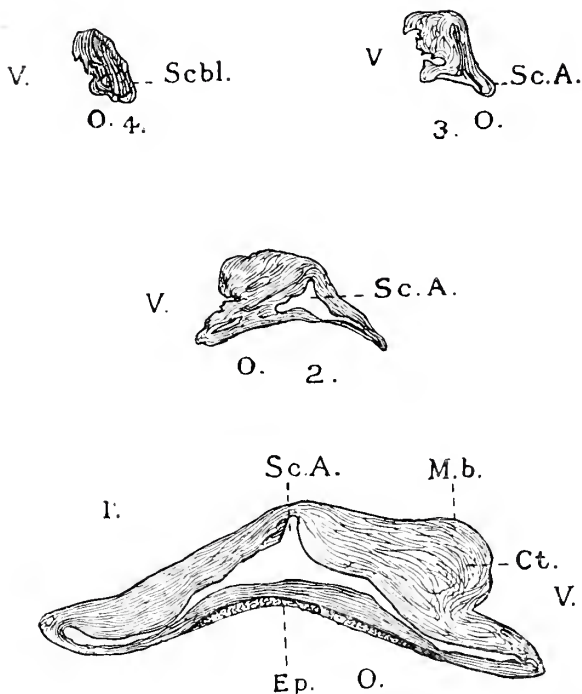
A few pigment-cells (*Pig.*) occur in the outer wall. The accessory scale (*Sc. a.*) is seen as a narrow strip of hyaline substance, running approximately parallel to the outer border of the lobe throughout the length of the latter. In sections from the same region of other salmon, there is sometimes a knotted swelling in that part of the accessory scale which lies within the broad portion of the lobe; it is more usual, however, to find this swelling nearer the distal end; such a condition is realised in fig. 6.

As the distal end is approached, the outline of the lobe and the shape of the accessory scale vary considerably in different salmon.<sup>1</sup> This is not surprising in an organ of such adventitious nature. The most constant and, moreover, the most striking feature of the distal area of the lobe is the presence of a patch of well-defined stratified epithelium. This epithelium, which is depicted in Text-fig. 2 (1), and under higher magnification in fig. 7 (*Ep.*), corresponds with the normal mucous epithelium of fishes; that is to say, it consists of round cells on the surface, passing into oval cells, and finally into palissade cells, the latter standing on a well-

<sup>1</sup> So far as I have observed they are always similar in both the adipose lobes of the same fish.

defined membrana basale (*M.b.*). Each cell contains a well-defined nucleus (*N.*). Serial sections show that this epithelium occupies an oval area a few millimetres from the tip of the lobe, and that it is confined to the outer vertical

TEXT-FIG. 2.



*T. Ss.* Adipose lobe of adult salmon. 1. In the region of the epidermis. 2. Slightly more than 3.5 mm. from the tip. 3. Slightly more than 1 mm. from the tip. 4. At the tip. *O.*, *V.* Outer, ventral aspects. *Ct.* Connective tissue. *Ep.* Epidermis. *M.b.* Membrana basale. *Sc. A.* Accessory scale. *Scbl.* Scleroblastic layer.

wall of the lobe. The invariable preservation of this epidermal tissue on a definite area, which is in no way specially protected, tends to suggest that the absence of epidermis from the remainder of the lobe is quite a normal

condition. Beneath the membrane basale (*M.b.*) of the outer wall are numerous small pigment-cells (*Pig.*); a few large isolated pigment-cells occur deep in the dorso-lateral surface. The connective tissue (*C.t.*) is more open in texture than that of the proximal region; the elongated nuclei of the cells (*N.*) are clearly depicted; towards the surface the nuclei are fewer and rounder. The accessory scale in this region is typically triradiate in outline as appears in Text-fig. 2 (1), with a swelling where the rays meet; but it is sometimes compressed into masses, which are plate-like in section and much vacuolated. The succeeding sections sketched in Text-fig. 2 (2, 3) show that the scale is continued to the extreme distal end of the adipose lobe, though in the last, No. 4, it only consists of a layer of scleroblasts (*Scbl.*), by means of which growth is continued throughout life.

#### (4) Examination of the Fat of the Adipose Lobe.

Sections from all parts of the lobe, taken from fresh specimens or from those which have been preserved in 10 per cent. formalin or in Müller's fluid, give the characteristic blackening on treatment with dilute osmic acid. This treatment is not entirely satisfactory, for while the fat-globules turn black, the connective tissue is also affected to a certain degree, turning brown, and the fatty matter is not sufficiently clearly differentiated. A better result is obtained by treating hand-sections, or frozen sections, with Sudan III according to the method described by Lee<sup>1</sup>; the fat-globules are then stained a deep orange colour, while the other tissues are scarcely affected. The distribution of the fat in a section prepared in this manner is illustrated in fig. 5. Nearer the distal extremity the fat is almost entirely confined to the inner side of the accessory scale; towards the base it spreads to the outer side, where it occurs in very considerable quantities.

The fact that a stain is readily obtained with Sudan III at

<sup>1</sup> 'Microtomist's Vade-Mecum,' ed. vi, p. 376.



the ordinary room temperature indicates that the fat is in a liquid condition, as opposed to a fluid crystalline condition; for the staining is consequent upon the solution of the dye in the fat, and the solubility is greater in liquid fats than in fluid crystalline, while in crystalline fats it cannot occur (until they are melted). The fat-globules are only slightly refractile. These observations suggest that the fat is allied to olein, but it is unlikely that the deposit is composed of any one pure compound.

A further examination of the fat was made by staining with Kultschitzky's hæmatoxylin after mordanting with bichromate of potash. The significance of this method in relation to fats has been explained in a paper by Lorrain Smith and W. Mair as follows:<sup>1</sup>

"Weigert's bichromate hæmatoxylin method for the staining of myelin has become firmly established in histology. On studying the effect of the bichromate mordant on fatty tissue we were convinced that the myelin method could be extended to apply to ordinary fats such as occur in fatty liver and fatty myocardium. This proved to be the case, and we found that the points on which the method depends are the length of time during which the bichromating is carried on, the strength of the solution, and the temperature at which the solution is applied. We early discovered that positive results could be obtained with formalin sections of fatty liver and heart if these were mordanted in a bichromate solution kept saturated at 37° C. After a fortnight of this treatment sections of fatty liver or heart yield extremely well defined and sharp blue staining of the fat-globules with Kultschitzky's hæmatoxylin followed by differentiation in Weigert's borax ferricyanide solution. On investigating the chemistry of this reaction Dr. Thorpe found that the process is due in the first place to the oxidising action of the bichromate. In the process of oxidation of a molecule of fat the oxide of chromium ( $\text{CrO}_3$ ) forms with it a compound which is practically insoluble in fat solvents. This

"Fats and Lipoids in Relation to Methods of Staining," 'Skandinavischen Archiv für Physiologie,' 1911, p. 251.

compound in virtue of the chromium oxide which it contains is able to form a lake with hæmatoxylin. It is necessary, however, to distinguish two kinds of fat. The fats in which no unsaturated grouping occurs are not acted on by the bichromate solution. On the other hand, where the molecule of fat contains such a group there occurs a slow process of oxidation, and it is while this oxidation is going on that the insoluble fat-chrome compound is formed. The blue substance which then results from staining is a threefold body composed of fat, mordant, and dye. It becomes clear, therefore, that only the unsaturated fats can be stained by this process, and that on account of the ease with which they can be oxidised by the bichromate. We found also that the method may be applied to the staining of lipoid bodies in which unsaturated groupings occur such as cholesterin and cerebrosides.

"In the next place it is interesting to find that as the bichromating goes on and the fat becomes fully oxidised and saturated a stage is reached at which no staining takes place. It is only during the process of oxidation that the chromium oxide combines with the unsaturated molecule in such a way that it can lake the hæmatoxylin. Olein, for example, when oxidised by bichromate of potassium yields finally dioxystearic acid, and this fat will not stain by these methods."

A number of sections of the adipose lobe 5 mm. thick, from a ripe female fish taken in the Wye nets, were prepared on the freezing microtome. These were then placed in a saturated solution of potassium bichromate and kept at a temperature of 36° C. In order to observe the effect of oxidation induced by this treatment, sections were removed at intervals of twenty-four hours, and subjected to the hæmatoxylin (Kultschitzky) test as described above. For two days no coloration took place; on the third day two minute blue specks were observed, showing that oxidation had begun, and on each of the succeeding days up till the eighth very few blue specks appeared. On the ninth day the first obvious blue coloration was noted; the next day the specks were fewer,

but darker in colour. This condition obtained till the nineteenth day, when the blue became more scarce again, indicating the approach of saturation. On the twentieth day, after which the observations were discontinued, the blue had almost disappeared.

A parallel series of observations was made with sections from the adipose dorsal fin of the same fish. In this case a distinct blue coloration was produced on the first day of bichromating, showing that oxidation had begun. The blue specks increased in number and in intensity of colour till the third day. The condition remained practically constant until the sixteenth day, when the colour became less intense and the specks fewer, indicating the approach of saturation. The fading continued until the twentieth, and last, day of the investigation.

While admitting that the foregoing experiments throw very little light, in the absence of other data, upon the chemical affinities of the fats under examination, they are highly relevant as emphasising the qualitative difference between them. We see, firstly, that the deposit in the adipose dorsal fin becomes oxidised much more readily than does that in the adipose lobe of the ventral fin; secondly, that the saturation point is approached at a correspondingly earlier date in the former than in the latter. We may, therefore, conclude that the fat in the lobe is of a more stable nature than that in the adipose dorsal fin.

### III. THE DEVELOPMENT OF THE ADIPOSE LOBE.

For the purpose of examining the details of the development of the adipose lobe I was fortunate enough to obtain a very complete series of young salmon, which were all hatched on the same day, and were removed from the water at intervals of a few weeks. The following table will show the age, size, and external appearance of the lobe in the eleven specimens which are to receive notice in this section :

No.	Age.	Length.*	Condition of adipose lobe.
1 .	5 weeks .	23.5 mm.	No trace externally
2 .	6 „ .	25 „	„ „
3 .	7 „ .	24 „	„ „
4 .	8 „ .	24 „	„ „
5 .	10 „ .	24.5 „	„ „
6 .	12 „ .	26.5 „	„ „
7 .	14 „ .	27 „	„ „
8 .	16 „ .	29.5 „	„ „
9 .	19 „ .	34.5 „	Very slight papilla
10 .	21 „ .	41 „	Slight projection
11 .	23 „ .	43.5 „	Distinct lobe

\* From tip of lips to fork of tail.

The specimens were fixed in Zenker's fluid. Transverse slices comprising the whole of the pelvic region were cut from each fish. These slices were embedded in paraffin, remaining in the oven for  $1\frac{1}{2}$  to  $3\frac{1}{2}$  hours, according to their size, and stained in hæmatoxylin (Grenacher<sup>1</sup>) on the slide.

#### (1) Description of the Sections.

In the youngest fishes examined (Nos. 1, 2 and 3) the splint makes its appearance in the series as an ossified strand, lying just beneath the surface in the upper angle of the fin-fold, and on a level with the plane which divides the two adductor muscles. Passing over a few sections in the posterior direction the head of the dorsal half of the first lepidotrich comes into view at the outer end of the basale and between the two adductor muscles; the splint in the same section is seen to be travelling ventralwards and outwards. Slightly beyond this again the splint is seen to come into close contact with the haft of the dorsal half of the first lepidotrich, so that the two together form a V-like structure of bone, lying in the dorsal region of the developing fin, with the angle of the V pointing towards the fin's proximal extremity. In the section last described the basale is still quite entire, and there is

<sup>1</sup> 'Practical Zoology,' Marshall and Hurst, ed. vi, p. 466.

not yet a trace of any of the other lepidotrichia, dorsal or ventral, in the region where the splint appears. This constitutes a marked difference from the condition in the adult fish, in which the junction of the splint with the dorsal half of the first lepidotrich occurs in the free portion of the fin outside the body-wall, and on a level with other lepidotrichia.

In No. 4 the splint is decidedly larger than in the foregoing specimens, and it is more curved. It first appears as a crescentic ossification placed nearly in the position noted above, but slightly higher up, for it subtends the adductor superior muscle. Further back, in addition to the portion which goes to meet the first lepidotrich, the upper extremity of the splint still remains in section as a disc of ossified tissue. No. 5 very nearly resembles No. 4, but in it the ventral half of the first lepidotrich appears in several sections before the splint finally disappears, a condition which is probably due to the greater extension of the fin (and consequently of the lepidotrichia) prior to sectioning, for it is not observed in the older and presumably more advanced specimens. In No. 5 is begun, and in Nos. 6 and 7 is continued, the blunting and obliteration of the primary fin-fold, which was so clearly defined in the younger specimens.

The body-scales are first clearly visible in No. 6. From No. 7 onwards there is an aggregation of connective tissue which forms a triangular area immediately above the fin; the base of the triangle is formed by the body-wall, and its acute apex points towards the division between the two adductor muscles. In No. 8 an abnormally large body-scale is found embedded in this triangular area, and this eventually becomes the accessory scale, the skeleton of the adipose lobe. In No. 9 the accessory scale is seen deeply embedded in the connective tissue of the body-wall at its basal anterior end; at the distal end it has grown towards the surface, and, pushing the body-wall before it, has formed a slight projecting papilla. The scale does not extend to the tip of the papilla.

The aggregation of connective tissue in which the accessory scale is embedded does not at this period reach the splint of bone which in the adult forms the support of the adipose lobe, which seems to indicate that the final condition is secondary. Not until No. 10 does the papilla lose connection with the body-wall on its inner aspect and become a free lobe.

In No. 11 the adipose lobe, though still relatively small, in outward appearance resembles the adult condition, only its outline is curved rather than angular. Vertical longitudinal sections of this specimen were prepared, and such a section containing the adipose lobe is depicted in fig. 8. The adipose lobe (*Ad.*) is seen lying in the angle between the body-wall and the ventral (pelvic) fin (*V. F.*). The epidermis (*Ep.*) has been very considerably damaged, but a trace of it still remains. A number of body-scales (*Sc.*) are seen, cut in various planes; they have not yet broken through their scale-pockets (*Sc. p.*) The accessory scale (*Sc. A.*) is cut somewhat obliquely, and is seen deeply embedded in the tissue of the adipose lobe; at this period it is scarcely distinguishable structurally from the normal body-scales, for it displays the characteristic thickened ridges, though in a much less marked degree. It is considerably longer than any of the normal body-scales; this does not appear in the drawing, but can easily be observed by following its course through the serial sections. Its proximal extremity, too, is much more deeply seated than that of the normal scales.

## (2) Summary of the Development and Mode of Growth.

In the course of development the formation of the bony splint, which connects the first fin-ray with the base of the adipose lobe occurs at the same time as that of the fin-rays—i. e. long before there is any trace of the body-scales. It is plainly visible in sections from Specimen 1 onwards. The body-scales do not appear till Specimen 6 is examined. Only in Specimen 8—that is to say, sixteen weeks after

hatching—is there any trace of the differentiation of the accessory scale, and the adipose lobe is not visible externally until the nineteenth week.

A scale, which is developed in a thickened area of connective tissue immediately above the base of the pelvic fin, is seen rapidly to increase in length, thenceforward gradually to lose its ridged markings and to become homogeneous in structure. This process begins about sixteen weeks after the hatching of the fish. As this specialised scale elongates it pushes before it the overlying tissues. First a ridge is formed in a horizontal direction along the body-wall; when the posterior extremity of the ridge reaches the space between the ventral fin and the body-wall, it leaves the latter and forms a slight projecting papilla. The papilla is at first conical, but, as the scale continues to grow, its outer aspect tends to become flat, the dorsal and ventral borders become sharply angular owing to the pressure of the edges of the growing scale, and the tissues on the inner aspect become largely adipose, in consequence of which its marginal walls fall inwards into folds along the lines of least resistance. Thus we arrive at the triangular outline of the adipose lobe which has been described in the adult salmon.

#### IV. OBSERVATIONS FROM OTHER TELEOSTEI.

(1) From the examination of other Teleostean fishes, and, where this was not practicable, from illustrations of such, it soon becomes evident that the occurrence of an enlarged scale at the outer angle of the base of the pelvic fin is a wide-spread feature of the order.

(2) The scale is constant for a given species.

(3) The scale is rarely absent from the Malacopterygian fishes, which are beyond doubt primitive Teleosteans, and is more constant in the less specialised forms in other groups.

(4) The scale is seldom seen in connection with ventrals which are thoracic in position, and never, so far as I am aware, with those which have reached the jugular position.



(5) So far as present observations go, the development of the accessory scale into an adipose lobe, possessing a skeletal connection with the ventral fin, is confined to the genus *Salmo*.

(1) Range of Fishes in which Scale has been  
Figured.

The following list of fishes in which the accessory scale is present is by no means complete, but will serve to indicate the wide range of its occurrence. I have not stated the groups from which it is absent unless I have actually observed this.

Sub-order—Malacopterygii.

Family Elopidae	. .	<i>Elops saurus</i> . Ox. 387, J. & E. 178. <i>Megalops atlanticus</i> . J. & E. 177, Camb. 547.
Hyodontidae	. .	<i>Hyodon tergisus</i> . J. & E. 180. <i>H. selenops</i> . J. & E. 181.
Albulidae	. .	<i>Albula conorhynchus</i> . Camb. 548.
Gonorhynchidae		<i>Gonorhynchus greyi</i> . Ox. 395.
Clupeidae	. .	None observed without.
Salmonidae	. .	Present in all genera except <i>Osmerus</i> , <i>Thaleichthys</i> , <i>Mallotus</i> and <i>Hypomesus</i> , which Boulenger re- gards as together forming a natural group. In <i>Salmo</i> is enveloped in connective tissue and largely sur- rounded by fat.

Sub-order—Ostariophysi.

Characiniidae	. .	<i>Hydrocyon goliath</i> . Camb. 578.
Cyprinidae	. .	<i>Carpiodes cyprinus</i> . J. & E. 71. <i>Cyprinus carpio</i> . Ox. 376. <i>Labeo falcifer</i> . Camb. 583.

<sup>1</sup> The references to figures are as follows. J. & E.: Jordan and Evermann, 'Fishes of North and Middle America,' vol. iv (plates), plate number. Camb.: 'Cambridge Natural History—Fishes,' page number. Ox.: 'A Treatise on Zoology,' pt. 9, Oxford, page number.

Siluridæ . . . *Trachinocephalus myops*. J. & E. 235.

. . . *Synodus fætens*. J. & E. 236.

Sub-order—Haplomi.

Scopelidæ . . . *Saurus undosquamis*. Gunther<sup>1</sup> 42.  
relatively enormous.

Sub-order—Percesoces.

Atherinidæ . . . *Atherina stipes*. J. & E. 332, long.  
thin scale.

*Kirtlandia vagrans*. J. & E. 336,  
very small.

*Atherinopsis californiensis*. J.  
& E. 341, very small.

Mugilidæ . . . *Mugil cephalus*. Ox. 420, J. & E. 343.  
*M. curema*. J. & E. 344, more  
marked than in *M. cephalus*.

*Chætomugil proboscideus*. J. &  
E. 346.

*Agonostomus monticola*. J. & E.  
347.

*Joturns pichardi*. J. & E. 348.

Polynemidæ . . . *Polynema quadrifilis*. Camb. 641.

Sub-order—Acanthopterygii (Division Perciformes<sup>2</sup>).

Berycidæ . . . *Beryx splendens*. Camb. 655.

Serranidæ . . . *Centropomus undecimalis*. J. &  
E. 476, indistinct.

*Hoplopagrus guntheri*. J. & E.  
513.

*Neomenis*. J. & E., present in all the  
species figured.

*Oxiurus chrysurus*. J. & E. 520.

*Rhomboplites aurorubeus*. J. &  
E. 521.

*Apsilus dentatus*. J. & E. 522.

*Verilus sordidus*. J. & E. 515,  
very small.

Acropomatidæ . . . *Xenocys jeisiæ*. J. & E. 526, very  
small.

<sup>1</sup> 'The Study of Fishes.'

<sup>2</sup> The accessory scale is not figured in any other division of the  
Acanthopterygii.

- Xenichthys agassizii*. J. & E. 527, very small.
- Pristipomatidæ *Hæmulon*. J. & E. 528-32, present in all species figured.
- Lythrulon opalescens*. J. & E. 536.
- Anisotremus surinamensis*. J. & E. 538, very small.
- A. bilineatus*. J. & E. 539, very small.
- A. virginicus*. J. & E. 540, more marked.
- Orthopristis chrysopterus*. J. & E. 541, very small.
- Microlepidotus inornatus*. J. & E. 542, very small.
- Sparidæ . . . Scale present in all Sparidæ figured.
- Mullidæ . . . *Mullus auratus*. J. & E. 360.
- Mulloidés rathburni*. J. & E. 361.
- Upeneus maculatus*. J. & E. 362.
- Pagrus auratus*. Camb. 665.
- Gerridæ . . . *Xystema cinereum*. J. & E. 556.
- Gerres olisthostomus*. J. & E. 557.
- Cyphosidæ . . . *Kyphosus sectatrix*. J. & E. 559.
- Hermosilla azurea*. J. & E. 559.
- Sciænidæ . . . *Cynoscion*. J. & E. 561-3. Present in all figured, but small.
- Sagenichthys ancylodon*. J. & E. 564, small.
- Bairdiella chrysura*. J. & E. 566, very small.
- Umbrina sinaloæ*. J. & E. 571, small.
- Menticirrhus americanus*. J. & E. 572, small.
- Pomacentridæ . . . *Dacyllus aruanus*. Ox. 443.
- Microspathodon chrysurus*. J. & E. 593.
- M. dorsalis*. J. & E. 594.
- Scaridæ . . . *Sparisoma hoplomystax*. J. & E. 611, very small.
- Scarus cozumile*. J. & E. 612, very small.

*Pseudoscarus guacamaia*. J. & E.  
617.

*Chætodontidæ* . *Chætodipterus faber*. J. & E. 619,  
small.

*Chætodon nigrirostris*. J. & E.  
620, doubtful.

## (2) Personal Observations.

Having noted the above-mentioned list of fishes in which the accessory scale is figured, I next proceeded to examine the actual nature of such a scale in various specimens of fish in the collection at Giessen.

The scale was first examined in fishes most nearly allied to the salmon, and the following observations were made :

(1) In all the species of *Salmo* the accessory scale is encased in an adipose lobe, and is connected at its base by a splint of bone with the outermost fin-ray of the pelvic fin.

(2) In other genera of the *Salmonidæ*<sup>1</sup> the accessory scale is well developed, but it is not enclosed in connective tissue.

(3) In other *Malacopterygian* fishes, especially in those which are adapted for active swimming, there is usually a marked accessory scale.

In *Clupea harengus* this scale is very elongated, moreover it is subtended along its inner margin by a strip of skin, so that it forms a hollow conical outgrowth from the body; there is no bony connection with the fin-rays. In *Hyodon* sp. (?) there is an elongated hollow scale, as in *Clupea*, but no trace of connective tissue.

Passing next to the *Ostariophysi*, various *Cyprinids* were eligible for examination. A well-marked accessory scale was found in *Abramis blicca*, *A. vimba*, *Squalius cephalus*, *Cyprinus* (*Leuciscus*) *dolula*, *Luciscus rutilus*, and *Chondrostoma nasus*; but in *Barbus vulgaris*, though distinct, it is very small.

<sup>1</sup> Except in *Osmerus*, and probably also in *Thaleichthys*, *Mallotus* and *Hypomesus*, but I have not had an opportunity to examine actual specimens of the last-named genera.

Some specimens of *Mugil cephalus* which had lain many years in spirit were the next that came to hand. In these a triangular patch of skin devoid of scales was found in the normal position of the accessory scale; there was no trace of a lobe.

A few genera of Acanthopterygian fishes in which the accessory scale has been figured were lastly examined. In these there was a small flap or thread of skin in the normal position, but the skin was entirely devoid of stiffening matter of any sort. Among the Mullidæ, *Mullus barbatus* and *M. furmutetus* showed such a condition; in the former, however, the thread of skin was very slender, and the latter was badly preserved, so that it was not possible to judge of its normal condition. Among the Sparidæ, *Sargus unimaculatus* shows a distinct flap of skin, but the occurrence of any projecting tissue is very doubtful in another species of *Sargus* (not identified), and the same must be said of *Charax puntazzo*. At all events there is in these forms a certain area at the outer angle of the ventral fin which is covered by skin, but is devoid of scales.

#### V. HOMOLGY AND FUNCTION.

In the foregoing pages we have examined the structure and the development of the adipose lobe of the pelvic fin of the salmon, and have seen from both these points of view that it resembles a body-scale. We have further noted that the presence of an accessory scale, or, in some instances, of a flap of skin in a corresponding topographical position, is a widespread feature of Teleostean fishes. That the adipose lobe is morphologically neither more nor less than a large body-scale which has never broken through its surrounding pocket is too obvious to require proof, but it would not be right to leave such a remarkable structure with no more than a platitude of this kind. The possibility of deriving the dermal fin-rays from body-scales, through the intermediary of a fin-like scale, such as we have in *Salmo*, occurred to my mind before my

attention was called to the admirable paper by Mr. Goodrich,<sup>1</sup> which deals with the same question from the developmental point of view. The following is a summary, in his own words, of Mr. Goodrich's observations :<sup>2</sup>

" Besides these body-scales are found scale-like exoskeletal elements set end to end in rows and forming jointed dermal fin-rays, called lepidotrichia, supporting the web of both the paired and the median fins. The minute structure of these fin-rays is almost or quite identical with that of the scales of the fish to which they belong. This is true more especially of the lower forms. In some, such as *Amblypterus*, there is a perfect gradation in form and arrangement between the body-scales and the fin-ray elements. But, as a rule, the transition is more abrupt, the segments of the rays acquiring a squarish or oblong shape, and not overlapping. Both the scales and the lepidotrichia are embedded in the dense connective tissue, the fibres of which enter the substance of the bone. Movable joints are formed by the fibrous matrix remaining unossified between them."

In the light of these facts, what had been but a passing idea acquired a real significance. The resemblance between a scale covered by connective tissue and a lepidotrich might easily be accounted for on grounds of analogy; but since we know that fin-rays are developed from scale-like elements, it seems just to regard the fin-like scale of *Salmo* as a connecting link between a body-scale and a lepidotrich.

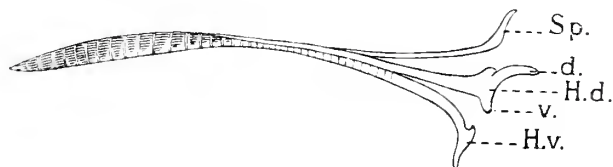
The fin-like nature of the adipose lobe is enhanced by the fact that it is connected by a splint of bone with the ventral fin. What, then, is the homology of this bony splint? We have noted that in the course of development it appears together with the lepidotrichia, which it resembles in structure, at a period before there is any trace of body-scales. Its position in the adult fish (p. 710), see fig. 2, indicates that it represents the dorsal half of an additional lepidotrich. If

<sup>1</sup> Goodrich, E. S., "On the Dermal Fin-rays of Fishes," 'Quart. Journ. Micr. Sci.' (v), vol. 47, 1903.

<sup>2</sup> 'A Treatise on Zoology,' Oxford, pt. ix, p. 212.

this homology is true, it should be noted at the same time that the ventral ramus of the head is wanting, as is also all trace of a ventral half; moreover, while the lepidotrichia are characteristically jointed, the splint is composed of a single piece.<sup>1</sup> These points are apparent in Text-fig. 3. It was further observed that the splint lies in a pocket of connective tissue, which in section resembles a scale pocket (see fig. 4, *Sp. h.*). The resemblance does not necessarily prove the homology of the two structures; it is merely satisfactory as not dispelling the idea. Taking the sum of these considerations we must suppose that we have to deal with the head

TEXT-FIG. 3.



The first (outermost) lepidotrich of the pelvic fin, together with the splint of bone which supports the adipose lobe, seen from the inner aspect. *Sp.* Bony splint (probably = dorsal half of a tenth lepidotrich). *H. d.* Head of dorsal half of first lepidotrich, with its dorsal ramus *d.*, and ventral ramus *v.* *H. v.* Head of ventral half of 1st lepidotrich.

and dorsal ramus of an additional lepidotrich which exhibits certain scale-like properties. Whether this lepidotrich is in the process of development or whether it is the vestige of a once fully developed ray I cannot at present decide with certainty, but its secondary fusion tends to show that it is vestigial. The exact homology of the adipose lobe itself is more obscure; that it arises as a scale we have seen. It is unlikely that it ever functioned as a fin-ray, or part of a fin-

<sup>1</sup> This is not necessarily a dissimilarity, for it will be noticed that the heads of the lepidotrichia are also devoid of joints, and the splint is only equal in size to the head of a fully developed Lepidotrich. The unjointed condition of the heads of the Lepidotrichia is, without doubt, due to secondary fusion.



ray, for in that case it would have reversed its course of evolution to a quite unimaginable extent. It is equally unlikely that it is a rudimentary fin-ray, or part of a fin-ray, since it contains but one large scale (instead of a series set end to end), and further, it lies dorsal to all parts of the fin skeleton. It is alienated from any fundamental resemblance with the adipose dorsal fin, in the first place because it develops comparatively late in the life of the fish, it is an adventitious outgrowth, that is to say, not the result of the development of a pre-existing embryonic fold (as is the case with the adipose dorsal); in the second place its fatty matter is of a different composition, and it is devoid of horny rays (actinotrichia). It seems probable, then, that the adipose lobe of the pelvic fin of *Salmo* is an organ *sui generis*. This does not detract, however, from its importance as suggesting the lines by which a fin may have been derived from a scaly outgrowth of the body-wall.

The function of this remarkable structure presents a puzzle to the investigator. It is almost impossible to believe that an organ of such large dimensions and regular occurrence in the genus *Salmo* serves no useful purpose in the daily round of these fishes. It seems justifiable to dismiss summarily the idea that it is a storage organ; the relatively stable properties of its fatty matter, the development of a stiffening axis, and its invariability in salmon of varying physical condition all point in this direction.<sup>1</sup> Again, as it is equally developed in both sexes, it is probably not analogous with the "claspers" of Elasmobranchs. Günther has laid stress on the value of the paired fins of fishes as balancing organs.<sup>2</sup> The pectorals and pelvics are placed where they are required to support the greatest weight of the fish on which they occur; thus the salmon, being thickly built in the posterior abdominal region, requires large ventrals. The adipose lobes may then act as additional balancing organs for the pelvic region; further, situated as they are just in the outer angle of the pelvic fin

<sup>1</sup> Except in one extreme case, see p. 709.

<sup>2</sup> 'The Study of Fishes,' p. 44.

(see fig. 1), they may act as dams to prevent the back-wash of water, which would be considerable in a fish with large pelvics, and so facilitate the swift motion through the water for which the salmon is noted.

### EXPLANATION OF PLATE 43,

Illustrating Mr. Edward W. Shann's paper, "An Account of the Anatomy and Homology of the Adipose Lobe of the Pelvic Fin of the Salmon."

#### LETTERING.

*Ad.* Adipose lobe. *B. m.* Basale metapterygii. *C. t.* Connective tissue. *Cu.* Cutis. *Ep.* Epidermis. *F. r. d.* Dorsal half of a fin-ray. *F. r. v.* Ventral half of a fin-ray. *Ft.* Fat-globules. *Ft.* Fatty tissue from which the fat has been extracted with alcohol. *M.* Muscle. *M. b.* Membrana basale. *N.* Nuclei. *Pig.* Pigment-cells. *Pt.* Pterygiophores. *Sc.* Body-scale. *Sc. a.* Accessory scale. *Sc. p.* Scale pocket. *Sp.* Splint of bone which supports the adipose lobe. *Sp. h.* Hole caused by the removal of the splint of bone which supports the adipose lobe. *V. f.* Ventral (pelvic) fin. *O., D., V.* Outer, dorsal, and ventral aspects.

Fig. 1.—A portion of the right side of a Wye salmon showing the pelvic fin; a piece of black pasteboard has been placed beneath the adipose lobe to render its outline more distinct. Photograph by Mr. J. T. Wadsworth, Manchester.  $\times \frac{2}{3}$ .

Fig. 2.—Skeleton of the right pelvic fin of a Wye salmon, seen from the ventral aspect; the rays are spread out and separated from the Basale metapterygii, with which in the natural condition they closely articulate. The numbers refer to the lepidotrichia. Photograph by Mr. A. W. Brown, St. Andrews. Slightly reduced.

Fig. 3.—Transverse section of the adipose lobe, about one third of the length from the basal end, together with the adjacent body-wall. From a Rhine salmon, whose adipose lobe measured 35 mm. by 6 mm. The sections, of which this is one, were block-stained iron-hæmatoxylin, and cut  $30\mu$  thickness.

Fig. 4.—Transverse section of the adipose lobe at the base (the lobe is quite continuous with the body-wall). Rhine salmon, dimensions not known. Method of preparation as above.

Fig. 5.—Transverse section of the adipose lobe in the middle region, to show the distribution of fat. ♀ fish from the Dovey; recently spawned, 18 or 19 lb., 35½ in. by 17¼ in.; shape very like a "fresh" fish. Not at all emaciated; no fat on the pyloric cæca; five years old. Hand section, stained Sudan III.  $\times 18$ .

Fig. 6.—Transverse section of the adipose lobe slightly distalwards from the middle. ♀ fish from the Wye, 29¾ in. by 15 in.; full of ripe eggs. No sign of emaciation; no fat on the pyloric cæca. Stained hæmatoxylin, cut 16  $\mu$  in thickness.

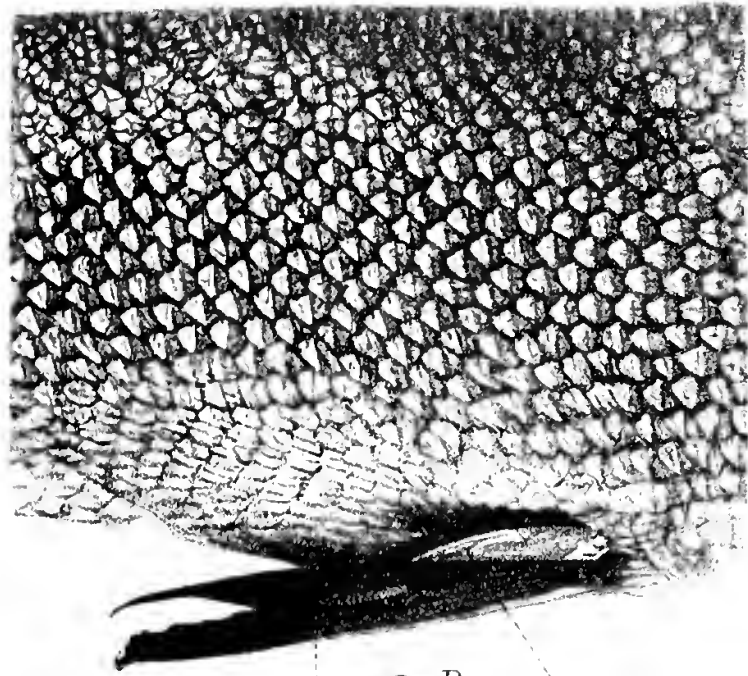
Fig. 7.—Transverse section of the outer wall of the adipose lobe near its distal end, to show epidermal epithelium. ♀ fish from the Wye, 35 in. by 19¼ in.; spring fish, unspawned. Great accumulation of fat on the pyloric cæca; nearly five years old. Prepared as above.

Fig. 8.—Vertical longitudinal section of the pelvic region of a young salmon. Age of fish twenty-three weeks; length, 43.5 mm. Stained hæmatoxylin, cut 12  $\mu$  in thickness.





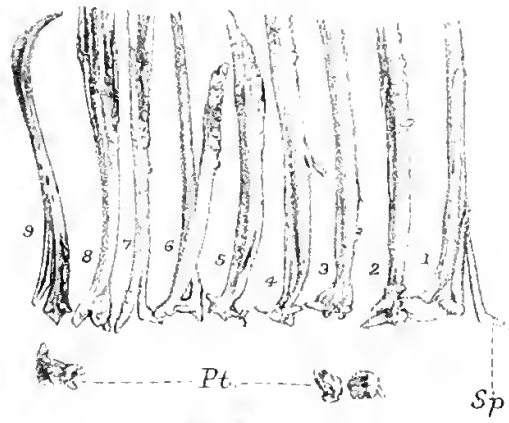




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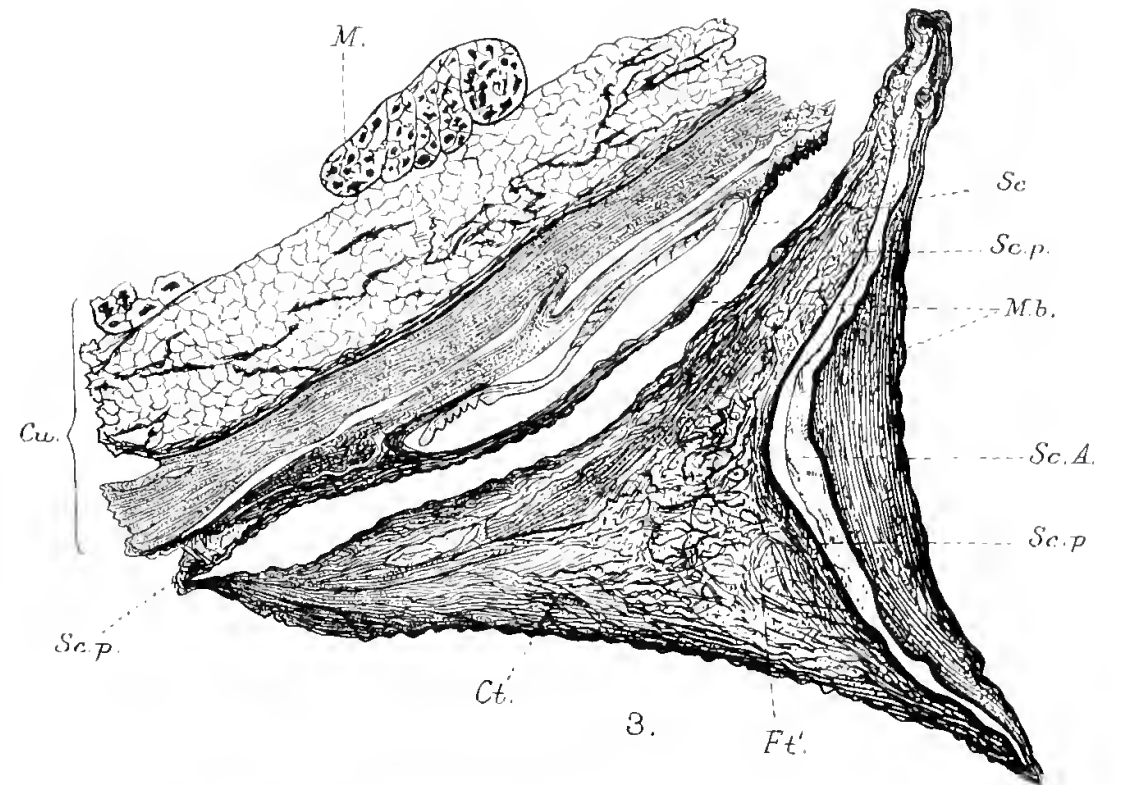
V.f. D. A.d.

Ct. M.b.



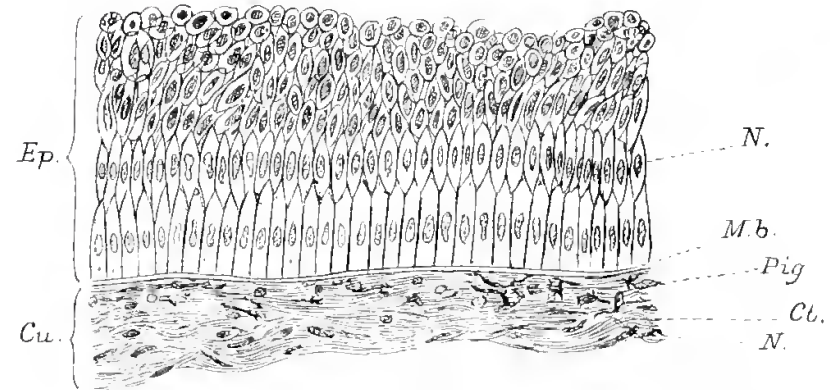
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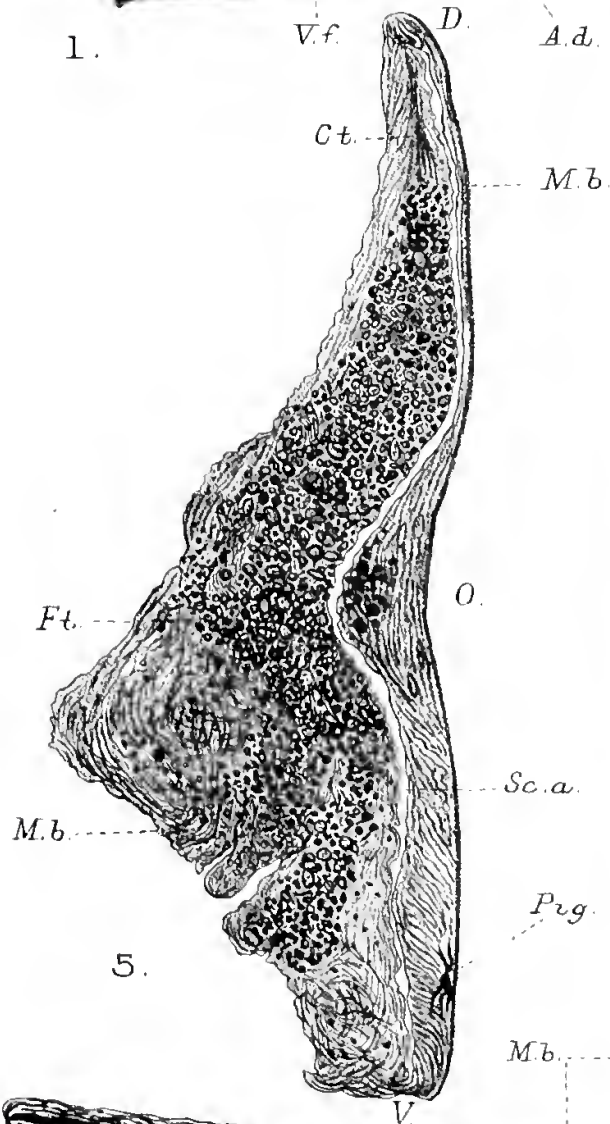


3.

Ft.



7.

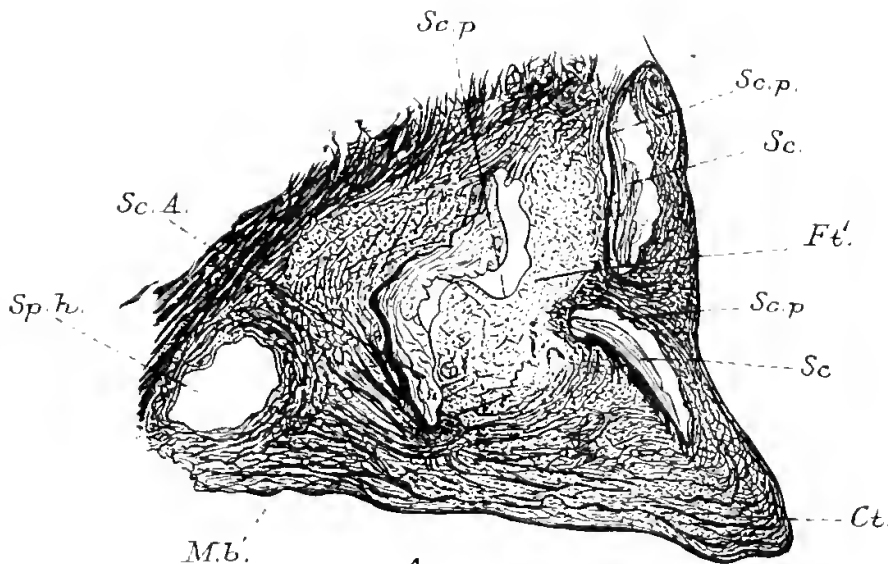


5.

M.b.

Pig.

M.b.



4.

M.b.

Sp.h.

Sc.A.

Sc.p.

Sc.p.

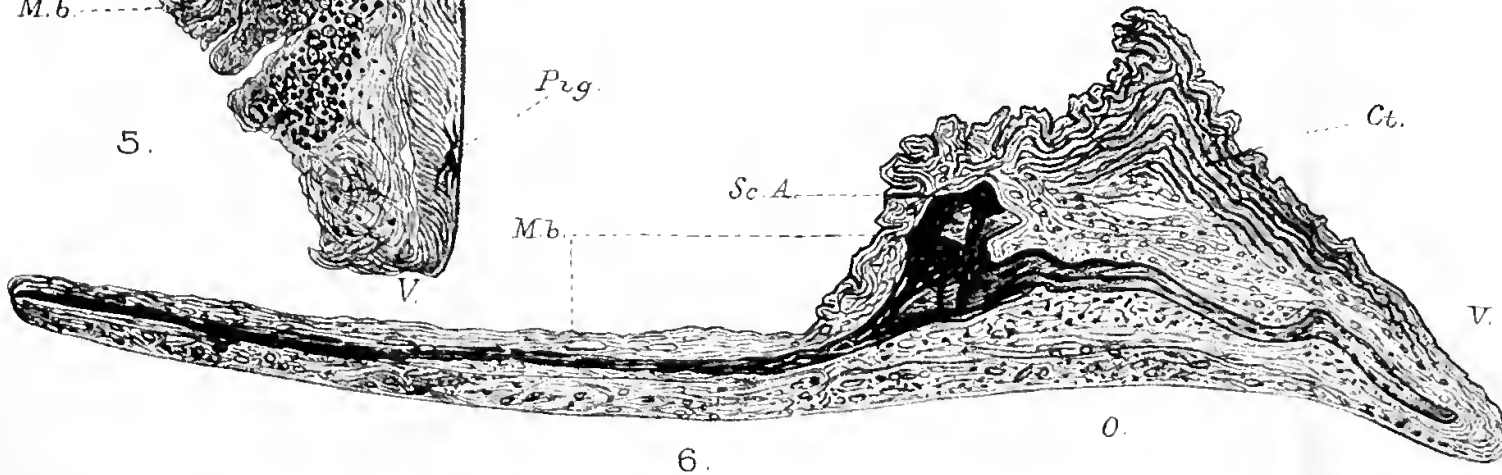
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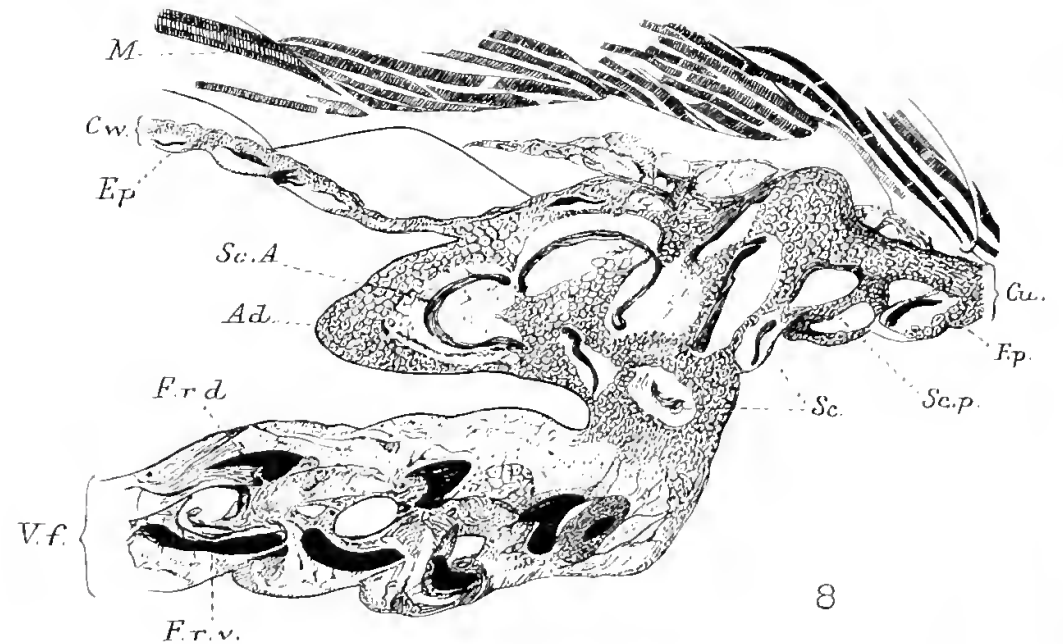
Ct.



6.

Sc.A.

Ct.



8.

V.f.

F.r.v.

F.r.d.

Sc.A.

Ad.

Ep.

Cw.

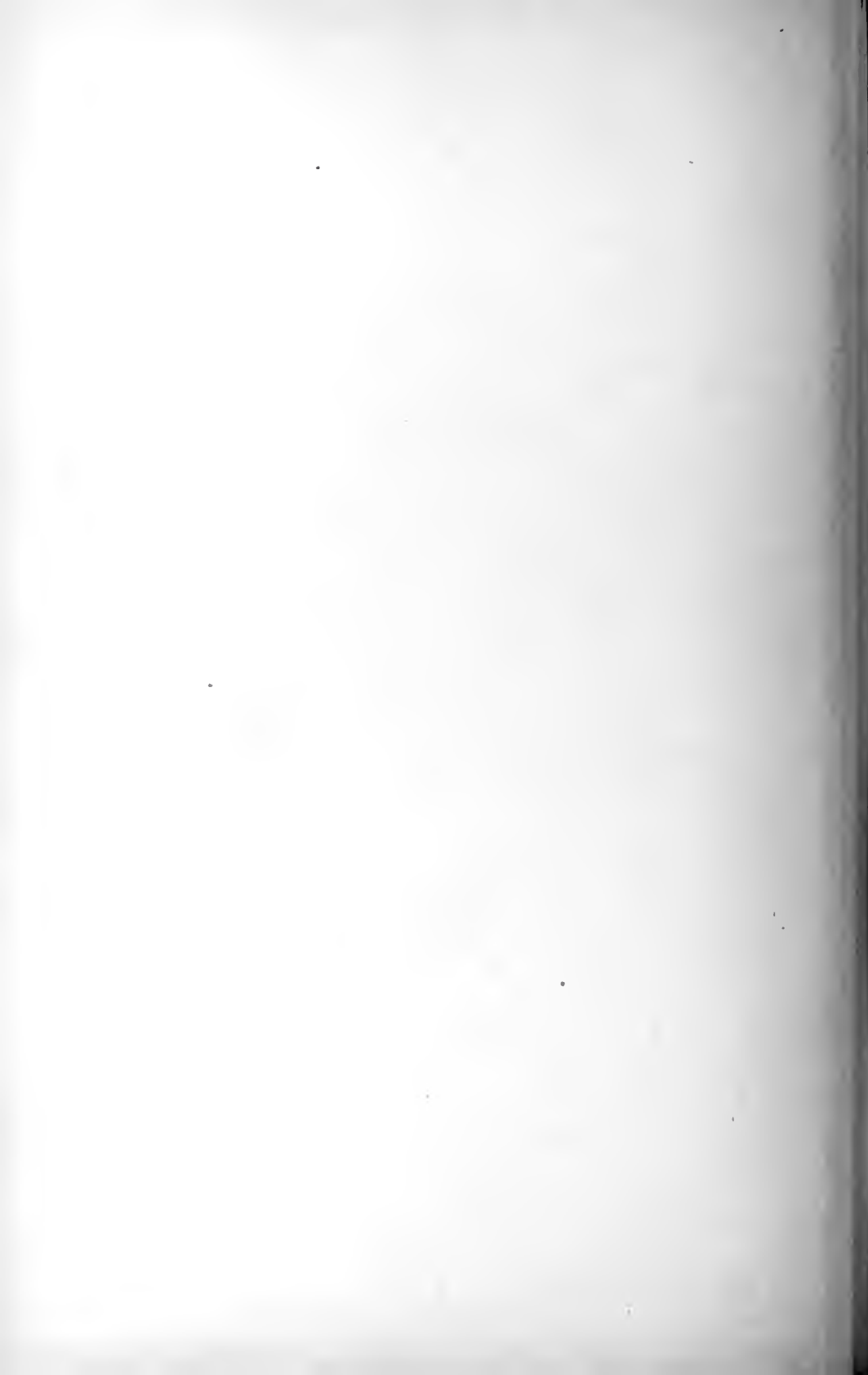
M.

Cu.

Fp.

Sc.p.







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EDITED BY

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CORRESPONDENT OF THE IMPERIAL ACADEMY OF SCIENCES OF ST. PETERSBURG, AND OF THE  
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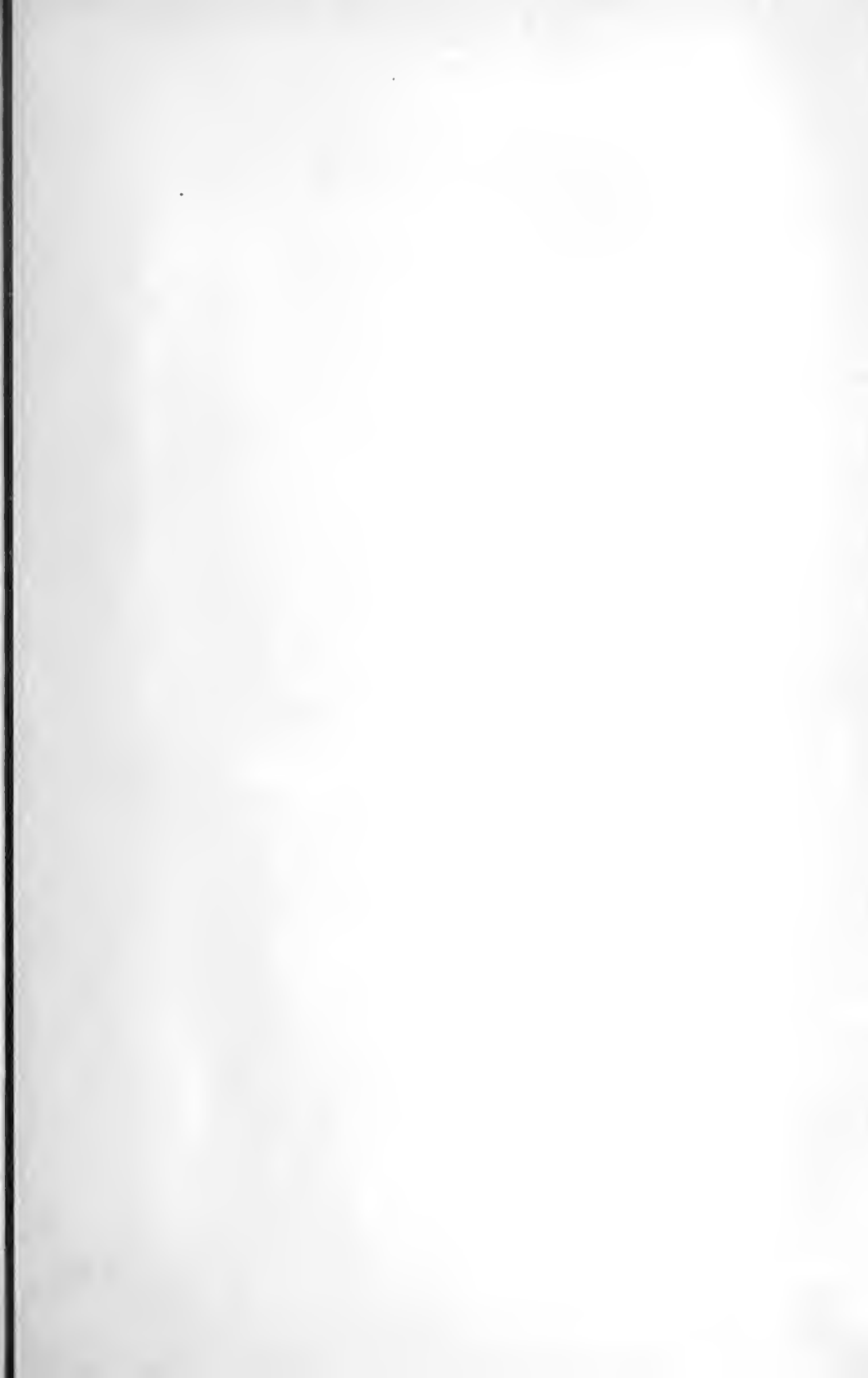
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